REMARKS

Amendments

Claims 1-21, 26 and 28-31 have been canceled, claims 22-25 and 27 have been amended, and claims 32-39 have been added. Upon entry of the amendment, claims 22-25, 27 and 32-39 will be pending. Support for the added claims can be found in the specification, for example, on page 6, lines 12-21, page 16, lines 9 through page 17, line 26; in Example 1; the Figures; and in the claims as originally filed.

The foregoing amendments are made solely to expedite prosecution of the application and are not intended to limit the scope of the invention. Further, the amendments to the claims are made without prejudice to the pending or now canceled claims or to any subject matter pursued in a related application. The Applicant reserves the right to prosecute any canceled subject matter at a later time or in a later filed divisional, continuation, or continuation-in-part application.

Rejections

Rejection under 35 U.S.C. § 112, first paragraph

Claims 22-27 stand rejected as allegedly failing to comply with the enablement requirement. The Examiner argues that the claim(s) contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Applicant respectfully traverses this rejection.

The Examiner questions the use of the mouse suspension model with the NTTP-1 knockout mouse for characterization of the role of NTTP1 gene in depression.

The Examiner suggests the mouse strains used in the creation of the NTTP-1 knockout mice are unsuitable for use in the mouse tail suspension model of depression. The Examiner cites Crawley *et al.*, Psychopharmacology, 132:107-124 (1997) extensively. Crawley *et al.*, 1997 provides comparison of inbred mouse strains in, and suggests suitability for, several behavioral phenotypes. Examiner paraphrases Crawley *et al.*, 1997:

> For example, two strains commonly used in ES cell and knockout generation C57BL/6 and various substrains of 129 are unusual on many standard behavioral paradigms. And

such unique traits of 129 and C57BL/6 mice are a problem for interpretation of behavioral phenotypes of null mutations.
(OA mailed 11/17/2004, page 3, lines 22-24 and page 4, line 1)

In the same reference, Crawley *et al.*, 1997 points out:

There is no best strain that can be recommended across all behavioral paradigms for all null mutations. Rather, the strain is chosen for its predicted sensitivity to the null mutation.
Crawley et al., 1997 page 120, paragraph 7.

In fact, throughout Crawley *et al.* "The 129 substrains and the C57BL substrains are emphasized..." (page 108, third paragraph). Crawley *et al.* recommends strain C57BL/6 for use in several behavioral tests including the complex learning Morris water task and contextual fear conditioning tests (page 110, Table 1 and paragraph 3). Crawley further states "The best choice of an inbred background on which to explore the impact of a null mutation on learning appears to be C57BL/6"(page 110, paragraph 3). In addition, "Strains with poor prepulse inhibition, including C57BL/6J..., can be used to improve prepulse inhibition" (as a model of schizophrenia; page 112, paragraph 6).

Although Crawley *et al.*, 1997 suggests suitable mouse strains for creation of knockout mice for use in several behavioral tests, Crawley makes mention of the mouse tail suspension test, nor any mouse model of depression, let alone strain suitability for those tests, rendering Examiner's point on strain suitability irrelevant for this test.

In fact, similar mouse strains used in the creation of the NTTP-1 knockout mice (i.e.129 ES cells and C57BL/6 mice), were used used by Heisler *et al.*, *Proc. Nat. Acad. Sci. USA* 95:15049-15054 (1998) to generate 5-HT_{1A} receptor-mutant mice. The homozygous 5-HT_{1A} receptor-mutant mice of Heisler *et al.* exhibited antidepressant-like responses in the six minute tail suspension assay.

The Examiner further questions enablement of the specification with respect to the mouse suspension model used with the NTTP-1 knockout mouse for characterization of the role of NTTP1 gene in depression. The instant application describes comparison of NTTP-1 phosphatase homozygous mutant mice (-/-) with age, sex and strain matched wild-type mice (+/+) in the six minute tail suspension test as a model of depression:

Specifically, homozygous mutant (-/-) male mice (n = 12) were more active during the six-minute tail-suspension test than their wild-type (+/+) male counterparts (n = 11),

resulting in a decrease of up to about 20%-50% in immobility time, suggesting that the mutants are less prone to depression-like behavior (i.e., an anti-depressive phenotype). On average, homozygous mutants had a total immobile time of 97.49 seconds, compared to 140.53 seconds for wild-type control mice, representing a decrease of about 30% in total immobile time.
(specification, page 48, lines 18-27)

One skilled in the art would recognize how to perform the tail suspension test as a mouse model of depression given both the specification, page 19, lines 20-29 and page 48 lines 18-27, and a plethora of available references (copies attached):

1. Ferrari, F., M. Cassinadri, P. L. Tartoni, and A. Tampieri. 1991. Effects of B-HT 920 in the tail-suspension test. Pharmacol Res 24:75-81;
2. Ferrari, F., and D. Giuliani. 1997. Effects of (-)eticlopride and 7-OH-DPAT on the tail-suspension test in mice. J Psychopharmacol 11:339-44;
3. Heisler, L. K., H. M. Chu, T. J. Brennan, J. A. Danao, P. Bajwa, L. H. Parsons, and L. H. Tecott. 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc Natl Acad Sci U S A 95:15049-54;
4. Nomura, S., H. Okada, R. Naruse, and K. Yamaoka. 1991. The tail suspension test for screening antidepressant drugs: comparison of movement in ICR and NMRI mice. Jpn J Psychiatry Neurol 45:113-4;
5. Porsolt, R. D., R. Chermat, A. Lenegre, I. Avril, S. Janvier, and L. Steru. 1987. Use of the automated tail suspension test for the primary screening of psychotropic agents. Arch Int Pharmacodyn Ther 288:11-30;
6. Steru, L., R. Chermat, B. Thierry, and P. Simon. 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85:367-70;
7. Steru, L., R. Chermat, B. Thierry, J. A. Mico, A. Lenegre, M. Steru, P. Simon, and R. D. Porsolt. 1987. The automated Tail Suspension Test: a computerized device which differentiates psychotropic drugs. Prog Neuropsychopharmacol Biol Psychiatry 11:659-71;
8. Thierry, B., L. Steru, P. Simon, and R. D. Porsolt. 1986. The tail suspension test: ethical considerations. Psychopharmacology 90:284-5;
9. Crawley "What's wrong with my mouse?: behavioral phenotyping of transgenic and knockout mice", Wiley-Liss, N.Y., N.Y. 2000 pages 194-195.

The six minute mouse tail suspension test was developed by Steru *et al.* twenty years ago as a new method for screening antidepressants in mice (Steru *et al.*, 1985). Mice, when suspended by the tail, will alternate between active attempts at escape and immobility. The tail suspension test was deemed an ethical improvement over another mouse "behavioral despair" test, the forced swimming test which involved placing the animal in a cylinder of tepid water (Thierry *et al.*, 1986). The mouse tail suspension test was automated and computerized in 1987 (Steru *et al.*, 1987). The mouse tail suspension test has been used to test several classes of antidepressant compounds: tricyclic antidepressants including Imiprimine, Desipramine, Amitriptyline, Imipramine methiodide (Nomura *et al.*, 1991); atypical antidepressants including Mianserin, Viloxazine, Nomifensine (Steru *et al.*, 1985); monoamine oxidase inhibitors including Clorgyline (Steru *et al.*, 1987); α_2-adrenoreceptor agonists including clonidine α_2-adrenoreceptor antagonists including yohimbine and the test drug "B-HT 920"(Ferrari *et al.*, 1991). The mouse tail suspension test was also used to implicate a dopaminergic abnormality in impaired central amine transmission underlying depression (Ferrari *et al.*, 1997).

In each cited reference above, the tail suspension test measured total immobility time over a period of six minutes as described in the application, page 48, lines 18-27. In fact, the mouse tail suspension model of depression is so well known in the art that commercially availabile equipment may be used to perform the test, for instance MED Associates MED-TSS-MS Complete single station tail suspension starter package; MED-TSS-300 Tail suspension add-on package and associated software SOF-821 Mouse Tail Suspension software (see e.g. www.med-associates.com).

Applicant submits that one skilled in the art would have been enabled by the specification to perform the mouse tail suspension test as a model of depression.

Withdrawal of the rejection is respectfully requested.

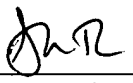
In view of the above amendments and remarks, Applicant respectfully requests a Notice of Allowance. If the Examiner believes a telephone conference would advance the prosecution of this application, the Examiner is invited to telephone the undersigned at the below-listed telephone number.

The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 13-2725.

Respectfully submitted,

1-13-05

Date



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EFFECTS OF B-HT 920 IN THE TAIL-SUSPENSION TEST

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SUMMARY

The effect of B-HT 920 on mice subjected to the tail-suspension test (TST), a behavioural tool for gauging depression, was studied. During the test, all the animals had bouts of agitation interspersed with periods of immobility but B-HT 920-treated animals exhibited a differentiated behavioural pattern according to the dose employed: at doses selective for dopaminergic (DA) autoreceptor stimulation (50 and 100 μg/kg), the periods of immobility were unaffected, while at higher doses (0.5, 1, 2 and 3 mg/kg), which are also considered to be active on α_2-adrenoceptors, they were much shorter, as they are when known antidepressants are administered. Both clonidine (75 and 150 μg/kg) and yohimbine (1 and 5 mg/kg), α_2-adrenoceptor agonist and antagonist, respectively, increased immobility time, while *N-n*-propylnorapomorphine, a DA-agonist, at a dose (10 μg/kg) specific for DA autoreceptors, was completely ineffective. Since the behavioural effects of B-HT 920 vary according to the dosage employed, discussion centres on what receptors might conceivably underly these effects and on their preclinical relevance.

KEY WORDS: B-HT 920, tail-suspension test, antidepressants, yohimbine, clonidine.

INTRODUCTION

Cardiovascular studies involving the use of B-HT 920 generally indicate that this substance mainly stimulates α_2-adrenoceptors [1]. It has also been reported as acting on the central nervous system as a selective D_2-DA autoreceptor agonist, given its ability, at low doses, to enhance synthesis of DA in the corpus striatum of mice following treatment with gamma-butyrolactone via a haloperidol-sensitive mechanism [2]. In accordance with biochemical findings, B-HT 920 was found to induce, in rodents, hypomotility, penile erections (PE) and stretching and yawning (SY) [3, 4], signs all considered indicative of an underlying D_2-DA stimulation [2, 5–7].

In particular, for PE and SY, a bell-shaped dose–response curve was obtained in

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that their stimulation was proportional to the dose up to the maximal active dose of 100 μg/kg and gradually decreased thereafter until it disappeared completely at 2 mg/kg, when even spontaneous PE and SY were suppressed [4]. It is therefore possible that different neurochemical mechanisms underly the diphasic effect of B-HT 920 on behaviour.

The present experiments were performed to investigate the activity of B-HT 920 on mice subjected to the tail-suspension test (TST), which is considered to be a behavioural tool for the study of depression, a pathology also associated with DA-ergic and NA-ergic dysfunctions.

TST in mice was recently proposed as a fairly selective, preclinical screening-tool for antidepressant drugs [8] and is a theoretical model conceptually similar to the forced swimming test [9] to which it owes its inspiration. They share the assumption that the immobility manifested by the animals in both experimental conditions reflects a state of depression. Accordingly, antidepressant agents are found to antagonize immobility in these tests, their effectiveness appearing to correlate quite well with their clinical potency. In view of the receptors stimulated by B-HT 920, clonidine (CLO), an α_2-agonist [10] and yohimbine (YOH), an α_2-antagonist [11], as well as a low D_2-DA 'autoreceptorial' dose of *N-n*-propylnorapomorphine (NPA), a well-known DA-agonist [12, 13], were assessed in the same experimental conditions. Moreover, for purposes of comparison, imipramine, a typical antidepressant agent, was also tested in our procedure.

MATERIALS AND METHODS

Subjects were male Swiss mice (35–40 g), housed in a temperature-controlled environment (21±1°C) on a 12 h light–dark cycle (from 7 a.m. to 7 p.m.), for at least one week before the experiments. At the moment of the tests they were naive to pharmacological and experimental experience.The tests were all performed between 10 a.m. and 2 p.m.

Tail-suspension test (TST) in mice

The test used, described by Steru *et al.* [8], is based on the assessment of the alternate episodes of agitation and complete immobility over a 6 min period. In brief, the mouse, suspended by the tail, makes apparent escape efforts; after several attempts it stops moving and hangs motionless. In our experimental conditions the mice were randomly selected in groups of four and submitted to three TSTs at regular 30 min intervals. In a first series of experiments saline was i.p. injected 25±2 min before one of the three tests, as shown in Table I. In a second series of experiments, untreated animals were submitted to the first test, subsequently saline or drugs were injected in the same animals 25±2 min before the second test and observations were made in the second and third tests. All experiments, performed by experienced researchers unaware of the drug treatment, were repeated at least twice so that no fewer than eight mice were employed for each treatment group.

Table I
Tail-suspension test (TST) in mice: effect of repetition and saline injection on immobility time (s)

Treatment (i.p.)	Test 1	Test 2	Test 3	
—	73.7±14.3	81.3±14.2	98.8±29.5	$F_{2,26}$=1.05; P=0.366
Saline A	62.2±15.2	79.5±21.5	113.7±18.0*	$F_{2,18}$= 4.2; P=0.032
Saline B	74.3±15.1	97.7±10.6	138.7±21.5**	$F_{2,14}$= 9.2; P=0.003
Saline C	69.8±21.3	100.9±20.7	92.4±13.2	$F_{2,14}$= 1.7; P=0.219

Each mouse was submitted to 3 TSTs at regular 30 min intervals. Mice were untreated, or injected with saline 25±2 min before the 1st, saline A, the 2nd, saline B, or the 3rd test, saline C.

Values are the mean ± SEM obtained from at least 8 animals per treatment group.

*P<0.05: saline A(3) versus saline A(1); **P<0.001: saline B(3) versus saline B(1) (ANOVA for repeated measures followed by Student's t-test for paired data).

Data analysis

The total immobility time for each 6 min period is presented in s as the mean ± SEM of the data from at least eight mice per treatment group. Analysis of variance (ANOVA) for repeated measures, Student's t-test for paired data or Student–Neumann–Keuls test (SNK) were used where appropriate. Probabilities below the 0.05 level were regarded as statistically significant.

Drugs

The following drugs were used: B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4*H*-thiazolo-4,5-*d*-azepine 2 HCl, Boehringer Ingelheim, Ingelheim am Rhein, Germany); *N*-*n*-propylnorapomorphine hydrochloride (Sterling Winthrop Research Institute, Rensselaer, NY, USA); yohimbine hydrochloride (Janssen, Beerse, Belgium); clonidine hydrochloride, Catapresan® (Boehringer Ingelheim, Ingelheim am Rhein, Germany); imipramine hydrochloride (Ciba-Geigy, Basel, Switzerland).

The substances were dissolved in distilled water and injected i.p. at a constant volume of 2 ml/kg; clonidine was supplied in solution by the manufacturers. Doses of drugs, referred to the weight of the salt, were chosen on the basis of previous experiments. Controls were given the same volume of saline and handled following the same procedure.

RESULTS

In a preliminary experiment (Table I), the behaviour of naive mice subjected to three TSTs at regular 30 min intervals was observed.

Despite a certain variability in the behaviour of mice, already noted by Borsini

in the forced swimming test [14], untreated animals, like those i.p. injected with saline 25 min before the first (saline A), second (saline B) or third (saline C) test, present alternate periods of motility and immobility, the latter tending to increase the longer the test continues. In our experimental conditions a significant level of difference was reached between the first and third tests in the case of saline A and saline B (Table I).

Figure 1 shows that B-HT 920 at 50 and 100 μg/kg, administered 25 min before the second test, did not affect immobility time with respect to controls; at higher doses (0.5, 1, 2 and 3 mg/kg), however, the animals exhibited almost uninterrupted motility throughout the two consecutive 6 min observation periods, with the result that total immobility time in each test was significantly different both from that recorded before drug treatment in the same animals and from that of mice injected with saline or B-HT 920 (50 and 100 μg/kg) at the same times. A similar result was obtained with a single acute dose of imipramine (15 mg/kg), which is reported for comparison. Table II reports the activity of CLO and YOH, α_2-adrenoceptor agonist and antagonist, respectively, and of NPA, an agonist at

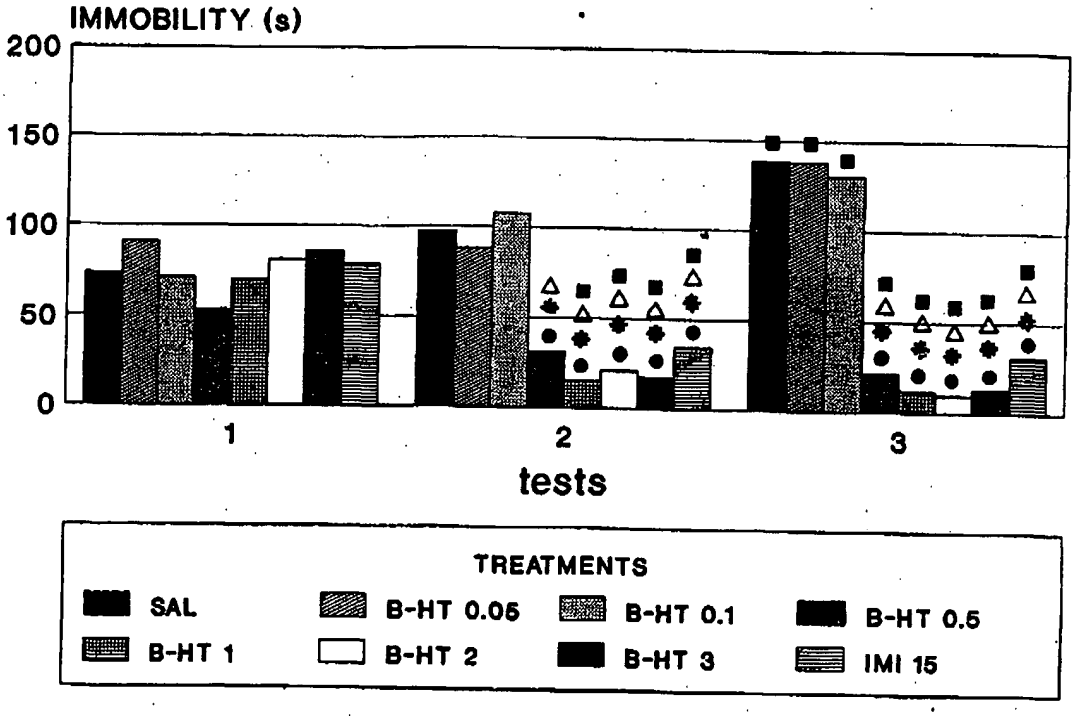

Fig. 1. Tail-suspension test (TST): influence of B-HT 920 on immobility time in mice. Each mouse was submitted to 3 TSTs at regular 30 min intervals. Mice were injected with B-HT 920 (B-HT, mg/kg i.p.) or imipramine (IMI, mg/kg i.p.) 25±2 min before the 2nd test. Each histogram represents the mean of 8 mice, standard errors have been omitted for clarity. They ranged between 5.8 and 20.6. ANOVA for repeated measures (one grouping and one within factor): Treatment: $F_{7,56}$=16.79; P<0.001; Test: $F_{2,112}$=6.78; P=0.0017(Greenhouse–Geisser prob.=0.002); Treatment× Test: $F_{14,112}$=6.59; P<0.001 (Greenhouse–Geisser prob.<0.001). ●P<0.05 with respect to saline in the same test (SNK test); ✱P<0.05 with respect to B-HT 0.05 in the same test (SNK test); ΔP<0.05 with respect to B-HT 0.1 in the same test (SNK test); ■ P<0.05 with respect to the values of the same animals in the first test (Student's t-test for paired data).

Table II
Effect of clonidine (CLO), yohimbine (YOH) and *N*-*n*-propylnorapomorphine (NPA) on immobility time of mice in the tail-suspension test

Treatment (*mg/kg i.p.*)	*Test* 1	2	3
Saline	73.7±15.1	97.7±10.6	138.7±21.5*
CLO, 0.075	90.9±17.5	180.0±22.2***	190.0±17.9*
CLO, 0.150	88.3±14.4	148.6±10.8*	161.0±22.2*
YOH, 1	71.4±12.7	152.9±10.5*	119.0±11.3*†
YOH, 5	78.1±15.1	119.6±25.3	179.4±9.6*†
NPA, 0.01	86.1±12.2	98.1±18.0	146.2±8.4*

Test procedures and modalities of injection as in Fig. 1.

ANOVA for repeated measures (one grouping factor and one within factor): Treatment: $F_{5,42}=1.73$; $P=0.149$; Test: $F_{2,84}=54.14$; $P<0.001$ (Greenhouse–Geisser prob. <0.001); Treatment×Test: $F_{10,84}=2.69$; $P=0.0065$ (Greenhouse–Geisser prob.=0.0079).

*$P<0.05$ with respect to the values of the same animals in the 1st test (Student's test for paired data); **$P<0.05$ with respect to the values of saline-treated animals in the second test (SNK test); †$P<0.05$ with respect to the values of the same animals in the second test (Student's test for paired data).

low doses of DA-autoreceptors [15], when assessed in the same experimental conditions. Both CLO (75 and 150 μg/kg) and YOH (1 and 5 mg/kg) increased immobility, while NPA (10 μg/kg) behaved similarly to saline treated animals.

DISCUSSION

Present work shows the effects of B-HT 920, yohimbine, clonidine and NPA in the TST test. The results obtained for B-HT 920 in this study are in line with those of previous reports regarding the different biochemical [2] and behavioural activities observed for low and high doses of the drug [3, 4, 16–18]. In fact, when it was injected at 50 and 100 μg/kg, doses which selectively stimulate D_2-DA autoreceptors, it was ineffective on the TST test; on the other hand, at 0.5, 1, 2 and 3 mg/kg, doses reputed to be active also or mainly on α_2-adrenoceptors [2], it potently antagonized immobility.

Since untreated mice, or those injected with saline, exhibited an increment in immobility after repetition of the test, and since immobility seems to reflect a depression-like state, B-HT 920, for a large dose range, can thus be said to demonstrate an antidepressant profile.

However, the results obtained with the other drugs tested in the same experimental conditions do not help to clarify completely the neurochemical mechanisms underlying B-HT 920-induced behavioural effects. In fact, while NPA, at a low dose selective for DA-autoreceptors, predictably failed to modify the mice's behaviour, CLO and YOH, α_2-adrenoceptor agonist and antagonist, respectively, both unexpectedly increased immobility time. Anomalous results for

B-HT 920 and CLO that are difficult to reconcile with α_2-adrenoceptor involvement have also recently emerged from cardiovascular experiments [1] and led the authors to put forward a hypothesis invoking the 'imidazole-preferring sites', for which CLO, at high doses, demonstrates affinity [19] and which could also be stimulated by B-HT 920. In fact, non-adrenergic binding sites, which display a high affinity for imidazole compounds and are therefore termed imidazole-binding sites, have recently been discovered in the brain [20]. Furthermore, an endogenous clonidine-displacing substance has been isolated in the bovine brain which is distinct from known neurotransmitters and modulators and which binds preferentially to IMID-binding sites and could represent its natural ligand [21].

In conclusion, B-HT 920 once again emerges as a new drug capable of strongly modifying animal behaviour. Its effects appear to vary according to the dosage employed but the present results do not provide conclusive evidence as to which receptors are responsible for mediating its potential antidepressant activity; moreover, they indicate the possibility that receptors other than α_2 are involved in its pharmacological activity.

ACKNOWLEDGEMENTS

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Effects of (–)eticlopride and 7-OH-DPAT on the tail-suspension test in mice

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The dopamine (DA) D_2/D_3 antagonist (–)eticlopride (0.02, 0.05 and 0.1 mg/kg), dose-dependently increased immobility in the mouse tail-suspension test. Chronic treatment with the same compound (0.05, 0.1 mg/kg, × 14 days) produced a different effect, decreasing immobility when animals were tested 24 h after the last injection. The DA D_3 agonist, 7-OH-DPAT, acutely administered before the same test, behaved biphasically, increasing and decreasing mice immobility at low (0.05 and 0.1 mg/kg) and high (1 and 2 mg/kg) doses, respectively. Chronically administered 7-OH-DPAT reduced the immobility time at 2 mg/kg but not at 0.1 mg/kg. These effects, coupled with measurements of locomotor activity and evaluation of mice behaviour in different conditions, are discussed in the light of putative DA involvement in depressive states and are considered as predicting antidepressant potential.

Key words: antidepressants; dopamine receptors; (–)eticlopride; 7-OH-DPAT; tail-suspension test

Introduction

Although the theory that the impaired central amine transmission underlying depression is dominated by a postulated noradrenaline and serotonin deficit (Feighner, 1983; Willner, 1991), there is growing support for the argument that a dopaminergic abnormality is crucially involved in this affective disorder (Post *et al.*, 1978; Chiodo and Antelman, 1980; Del Zompo *et al.*, 1984). Impaired dopamine (DA) transmission, at least in some kinds of depression, is suggested both by the indirect clinical evidence that decreased dopaminergic activity in Parkinson's disease is often associated with depression (Taska and Brodie, 1983) and by the diminished DA turnover found in a subpopulation of depressed patients (Post *et al.*, 1978). Moreover, antidepressant-like effects are produced by different compounds and treatments that enhance dopaminergic transmission: the DA uptake inhibitor, nomifensine (Feighner, 1983), electroconvulsive shock (Hutchinson and Smedberg, 1963) as well as some DA agonists such as bromocriptine (Waehrens and Gerlach, 1981) or piribedil (Post *et al.*, 1978), have all been reported to be effective as antidepressants. The efficacy as an antidepressant of the DA D_2 antagonist, sulpiride, at low doses (Benkert and Holsboer, 1984) has been ascribed to the preferential blockade of presynaptic DA D_2 autoreceptors leading to a stimulation of DA function (Serra *et al.*, 1990). Accordingly, we investigated the effects exerted by (–)eticlopride, a DA D_2/D_3 antagonist belonging to the benzamide class (Baldessarini *et al.*, 1993; De Paulis *et al.*, 1985; Hall *et al.*, 1985), in the tail-suspension test in mice, a behavioural screening test for antidepressants (Steru *et al.*, 1985). The drug was found to be active at very low systemic doses (Ferrari and Giuliani, 1996), thus distinguishing it from (–)sulpiride which has a low ability to cross the brain barrier (Hall *et al.*, 1985). The tail-suspension test (Steru *et al.*, 1985) shares with Porsolt's forced swimming test (Porsolt *et al.*, 1979) the ability to induce a state of immobility, sometimes referred to as 'behavioural despair', in animals; this is claimed to reproduce a condition akin to human depression (Willner, 1984, 1991). Although some reports have questioned the specificity of 'behavioural despair', of the animal models of depression, it has been considered not only to be reliably predictive but also to possess a certain face validity, owing to the analogy between the human state and the reluctance of animals to react to stressful situations, manifested by total immobility (Willner, 1984).

As chronic treatment is usually necessary for antidepressants to have any clinical effect, the tail-suspension test was performed after both acute and chronic (daily for 15 days) injections of (–)eticlopride. Furthermore, to test the hypothesis that a stimulation of DA receptors might be involved in improvement in a 'depressive-like state' (Spyraki and Fibiger, 1981; Borsini *et al.*, 1985; Serra *et al.*, 1989), we investigated the effects exerted in the tail-suspension test by acute and chronic 7-OH-DPAT, a DA agonist that has considerably higher selectivity for D_3 than for D_2, D_4 and D_1 receptors (Levesque *et al.*, 1992). The DA D_3 receptor has aroused great interest because its preferential localization in the limbic system of rodents and humans points to a key role in several affective disorders (Sokoloff *et al.*, 1990; Levesque *et al.*, 1992). In view of a possible relationship between the effects of (–)eticlopride and 7-OH-DPAT on immobility in the tail-suspension test and on general mice behaviour, additional experiments were performed to evaluate the influence of the two drugs on locomotor activity and of 7-OH-DPAT on grooming and sedation in animals.

Materials and methods

The subjects were male Swiss mice (30–35 g, Harlan–Nossan, Udine, Italy) housed in a temperature-controlled environment (21 ± 1°C) on a 12 h light : dark cycle (lights on 07.00 to 19.00 hours) for at least 1 week prior to the start of the experiments. All the tests were performed between 09.00 and 13.00 hours in a soundproof, air-conditioned room, temperature, 20 ± 2°C. The mice were monitored by experienced observers unaware of the experimental design. The experiments followed the European Community regulations on the care and use of animals for scientific purposes (CEE Council 86/809 and Italian D.L. 27-01-92, No. 116).

Tail-suspension test

The procedure used, described by Steru *et al.* (1985), is based on the assessment of alternate episodes of agitation and complete immobility over a 6-min period. In brief, the mouse, suspended by the tail, makes apparent escape efforts; after several attempts it stops moving and hangs motionless. The measurement of total immobility time (sec) was performed under blind conditions with a stopwatch. A preliminary experiment conducted in two consecutive tests at 30-min intervals showed that, despite a certain variability in animals' behaviour, each mouse exibited a fairly consistent profile with a non-significant increment of immobility, saline injection did not modify this pattern. We decided to modify slightly Steru's original procedure performing two consecutive tests on the same animal on the same day.

Acute treatment

On Day 1, all naïve animals were first submitted to the tail-suspension test before any treatment was administered. They were divided into groups (at least eight mice in each) which were not significantly different with regard to the behaviour in question (first test, Day 1). They were treated immediately as follows: saline, (–)eticlopride (0.02, 0.05 and 0.1 mg/kg) or 7-OH-DPAT (0.05, 0.1, 1 and 2 mg/kg) and returned to their home cages. They were again submitted to the tail-suspension test 25 min later (second test, Day 1).

Chronic treatment

The same groups of animals injected with (–)eticlopride at 0.02, 0.05 and 0.1 mg/kg or 7-OH-DPAT at 0.1 and 2 mg/kg, were treated once daily for 14 consecutive days. On the fifteenth day they were tested again 24 h after the last treatment (first test, Day 15), immediately injected with the drugs and retested after a 30-min interval (second test, Day 15).

Locomotor activity

Acute treatment (Table 1, A)

Experimentally-naïve mice were randomly divided into six groups that were treated with saline, (–)eticlopride at 0.02, 0.05 and 0.1 mg/kg or 7-OH-DPAT at 0.1 and 2 mg/kg; 25 min later, the animals were placed in groups of three (with the same treatment) in special motility cages (Cibertec, Spain) (25 × 45 cm), equipped with a photoelectric barrier in the floor, connected to a digital counter. Locomotor activity was automatically recorded for 30 min, each locomotor movement corresponding to a single count; the apparatus did not record the movements of the tail and other small amplitude movements. At least four experiments were performed per dose.

Chronic treatment (Table 1, B and C)

Experimentally-naïve mice were randomly divided into five groups that were chronically treated with saline, (–)eticlopride at 0.05 and 0.1 mg/kg or 7-OH-DPAT at 0.1 and 2 mg/kg following the same schedule as for mice used in the tail-suspension test (once daily for 14 consecutive days). On the experimental day, 24 h following the last treatment, each group was randomly divided into two subgroups; the first was untreated (Table 1, B), the second received a further injection of the chronically-administered drug at the same dose (Table 1, C) before the assessment of locomotor activity. The treatment times and procedures were the same as for acute treatments.

Grooming and sedation

Acute treatment

Experimentally-naïve mice were placed singly in glass observation cages (25 × 25 × 34 cm); after a 25-min acclimatization period they were injected s.c. with saline or 7-OH-DPAT (0.1 and 2 mg/kg) and immediately observed for 50 min. Grooming was evaluated according to Gispen *et al.* (1975). In brief, an observer recorded every 15 sec whether or not the mouse displayed the phenomenon defined as face or body washing, scratching or licking paws or tail. If one of these signs was observed a positive score was given. To measure sedation, each mouse was monitored for 30 sec at 5-min intervals throughout the test period, starting at time 0. Sedation was scored as 0 = absent, 1 = immobile for at least 15 sec with open eyes and 2 = totally immobile for at least 25 sec. The values for each mouse represent the sum of the scores for each parameter during the test period.

Chronic treatment

The same groups of animals treated with saline or 7-OH-DPAT 0.1 and 2 mg/kg were injected once daily for 15 consecutive days. On the fifteenth day, 25 min after the last injection each treatment group was assessed for grooming and sedation. The procedure was the same as for acute treatments.

Drugs and treatments

The following substances were used: (–)eticlopride, S(–)-3-chloro-5-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride] and 7-OH-DPAT, ±-7-hydroxy-dipropylaminotetralin hydrobromide (RBI, USA). All the drug solutions were freshly prepared and the substances were dissolved in saline at a concentration that allowed the administration of 1 ml/kg, s.c.

Statistical evaluation

The data are presented as means (± SEM) and were analysed using the ANOVA followed by the Student–Newman–Keuls (SNK test), Student's *t* and Kruskal–Wallis tests. The Mann–Whitney U-test was used where appropriate, with the level of significance set at $p < 0.05$.

Results

Figure 1 shows that naïve mice subjected to the tail-suspension test (first test, Day 1) exhibited periods of immobility and a similar behavioural pattern occurred in the same animals when the test was repeated 25 min after saline injection (second test, Day 1). (–)Eticlopride (0.02, 0.05 and 0.1 mg/kg) administered acutely before the second test, dose-dependently increased the immobility time of mice with respect to their baseline; immobility induced by (–)eticlopride at 0.05 and 0.1 mg/kg significantly differed from that of controls in the same test [$F(3,36) = 30.7$; $p = 0.001$]. When tested again, 24 h after the last injection of chronic drug treatment (first test, Day 15), saline- and (–)eticlopride 0.02 mg/kg-treated animals did not differ in their behaviour with respect to their first test exposure (Day 1, first test), despite a slight increment in immobility recorded in both treatment groups. On the other hand, as shown in Fig. 1, mice treated chronically with (–)eticlopride at 0.05 and 0.1 mg/kg exhibited reduced immobility periods so that their immobility time was significantly lower not only than that of chronically saline-treated animals [$F(3,36) = 4.4$; $p = 0.009$] but also than that of the same animals in the first test at Day 1. When the second test, Day 15 (Fig. 1), was repeated after a further injection of the drugs, a significant increase in immobility with respect to the first test, in the same day was detected in (–)eticlopride 0.05 and 0.1 mg/kg-treated mice, but the effect was less marked than that obtained after the first acute injection at the same doses (second test, Day 1).

Figure 2 shows the effects exerted by acute and chronic 7-OH-DPAT in the tail-suspension test; the first day the drug (0.05, 0.1, 1 and 2 mg/kg) behaved biphasically, potently increasing and decreasing mice immobility at the two lowest and highest doses, respectively [$F(4,37) = 53$; $p = 0.001$]. After chronic treatments, a dose of 0.1 mg/kg did not change the immobility time, thus differing from the first treatment while a dose of 2 mg/kg abolished immobility [$F(2,15) = 9.2$; $p = 0.02$].

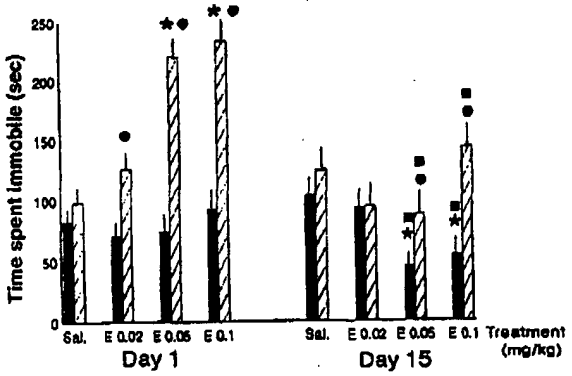

Figure 1 Tail-suspension test: influence of (–)eticlopride on immobility time in mice. Each histogram is the mean ± SEM total duration of immobility during a 6-min test. Immobility was assessed before (black bars) and 30 min (hatched bars) after s.c. injection of saline (Sal.) or (–)eticlopride (E). The same animals in each treatment group were tested on Day 1 and Day 15, after 14 daily treatments (for details see Methods). *Significantly different from animals treated with saline on the same day and in the same test (ANOVA followed by Student–Newman–Keuls test). ● Significantly different from the same group of animals before treatment, (Student's *t*-test). ■ Significantly different from the same group of animals on Day 1 in the same test (Student's *t*-test)

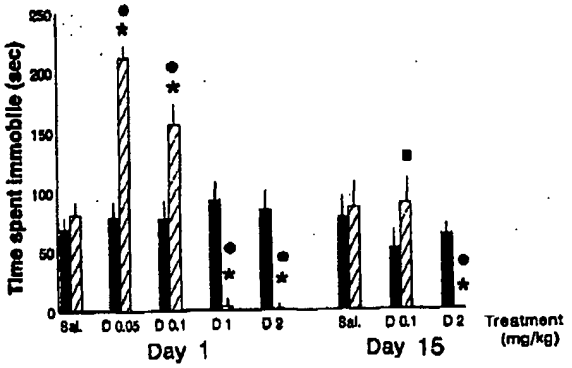

Figure 2 Effects of 7-OH-DPAT in the tail-suspension test in male mice. Each histogram is the mean ± SEM total duration of immobility during a 6-min test. Immobility was assessed before (black bars) and 30 min (hatched bars) after s.c. injection of saline (Sal.) or 7-OH-DPAT (D). The same animals in each treatment group were tested on Day 1 and Day 15, after 14 daily treatments (for details see Methods). *Significantly different from animals treated with saline on the same day and in the same test (ANOVA followed by Student–Newman–Keuls test). ● Significantly different from the same group of animals before treatment, (Student's *t*-test). ■ Significantly different from the same group of animals on Day 1 in the same test (Student's *t*-test)

Table 1 shows that acute treatment with (–)eticlopride at 0.05 and 0.1 mg/kg and 7-OH-DPAT at 0.1 and 2 mg/kg significantly reduced mice motor activity (A) [$F(3,14) = 37.9$; $p < 0.001$; $F(2,11) = 14.8$; $p < 0.001$, respectively]. Mice chronically treated with (–)eticlopride and 7-OH-DPAT at these doses and tested 24 h after the last injection did not differ in motor activity from saline-treated animals (B). Acute injection with (–)eticlopride in chronically-treated mice (C) reduced their locomotor activity with a pattern similar to that obtained after the first injection [$F(2,11) = 129.3$; $p < 0.001$]; acute 7-OH-DPAT (C) inhibited the locomotor activity only at the lower dose [$F(2,11) = 29.5$; $p < 0.001$].

Figure 3 reports the effects induced by acute and chronic 7-OH-DPAT (0.1 and 2 mg/kg) on mice grooming and sedation. Whereas grooming was always potently reduced by the drug, sedation was increased by both doses after acute injection and only by the lower dose after chronic injection.

Discussion

Despite some disagreement about the validity of animal models that both mimic affective disorders and are selectively sensitive to clinically useful drugs (Betin *et al.*, 1981; Willner, 1984; Borsini *et al.*, 1985) the tail-suspension test, like the forced swimming test, seem to be a fairly reliable indicator of potential antidepressant agents (Porsolt *et al.*, 1979; Willner, 1984; Steru *et al.*, 1985). The immobility induced in animals by both procedures is reduced by typical and atypical

Table 1 Effect of acute and chronic (–)eticlopride and 7-OH-DPAT on mice locomotor activity

Groups	Treatment (mg/kg, s.c.)		Locomotor activity
	Chronic	Acute	(counts)
A	—	Sal.	3322±110
A	—	Eti, 0.02	2937±318
A	—	Eti, 0.05	1006±190*
A	—	Eti, 0.1	972±229*
A	—	D, 0.1	2064±66*
A	—	D, 2	1814±415*
B	Sal.	—	3497±111
B	Eti, 0.05	—	3619±119
B	Eti, 0.1	—	3683±103
B	D, 0.1	—	3320±115
B	D, 2	—	3535±120
C	Sal.	Sal.	3482±137
C	Eti, 0.05	Eti, 0.05	1041±154*
C	Eti, 0.1	Eti, 0.1	861±86*
C	D, 0.1	D, 0.1	2123±102*
C	D, 2	D, 2	3298±131†

Each number is the mean ± SEM of at least four experiments. Acute treatments (A and C) with saline (Sal.), (–)eticlopride (Eti) or 7-OH-DPAT (D) were performed 25 min before locomotor assessment (30 min). In the case of chronic treatments alone (B), the experiments were performed 24 h after the last injection of the drugs. *Significantly different from respective saline-treated animals (ANOVA followed by Student–Newman–Keuls test). †Significantly different from the acutely-treated animals (A) at the same dose.

antidepressants, electroconvulsive shock, REM sleep deprivation and enrichment of the environment. When coupled with measurements of locomotor activity in different conditions, these tests can distinguish drug locomotor stimulant or reducing effects from antidepressive- or depressive-like effects (Porsolt *et al.*, 1979; Borsini *et al.*, 1985). The results obtained in the two experimental models are in agreement, however, some procedural advantages of the tail-suspension test over the forced swimming test and the avoidance of hypothermic stress associated with immersion in water (Steru *et al.*, 1985), prompted us to choose the tail-suspension test for our experiments.

Our results show that, as has been reported in the swimming test (Porsolt *et al.*, 1979), immobility in mice submitted to the tail-suspension test is a behavioural parameter that is very sensitive to activity on central DA systems. The effects may differ, depending on whether the drug is administered acutely or chronically; in our study, acute (–)eticlopride in common with other DA antagonists tested previously (Porsolt *et al.*, 1979; Borsini and Meli, 1988) increased mice immobility, this effect was seen to be significantly diminished after chronic treatment. What seemed to us to be particularly interesting was that mice chronically treated with 0.05 and 0.1 mg/kg and tested 24 h after the final injection (first test, Day 15), exhibited extremely reduced episodes of immobility. This is in accordance with the data of Del Zompo *et al.* (1984) who reported an antidepressant effect of withdrawal from chronic neuroleptic treatment. Locomotor activity assessed in mice chronically treated with (–)eticlopride at 0.05 and 0.1 mg/kg was found to be unchanged with respect to controls; moreover, acute injection at the same doses in chronically-treated mice behaved similarly to acute injection in naïve mice. These data, therefore, suggest that the results obtained after (–)eticlopride in the tail-suspension test are not related to an aspecific effect on animal locomotor activity.

The acute D_2/D_3 DA agonist 7-OH-DPAT behaved biphasically, potently increasing and decreasing mice immobility at low and high doses, respectively. To the best of our knowledge, 7-OH-DPAT effects in the tail-suspension test are a new finding. In previous work, DA agonists at low dosages, reputed to be selective for DA D_2 autoreceptors and, accordingly, found to be sedative, failed to modify immobility time either in the tail or swimming tests (Porsolt *et al.*, 1979) and at high postsynaptic doses decreased the same parameters (Steru *et al.*, 1985; Borsini and Meli, 1988). It must be pointed out that, whereas, in the case of the dopaminomimetic drugs tested in the past it was difficult to establish their antidepressant-like profile,

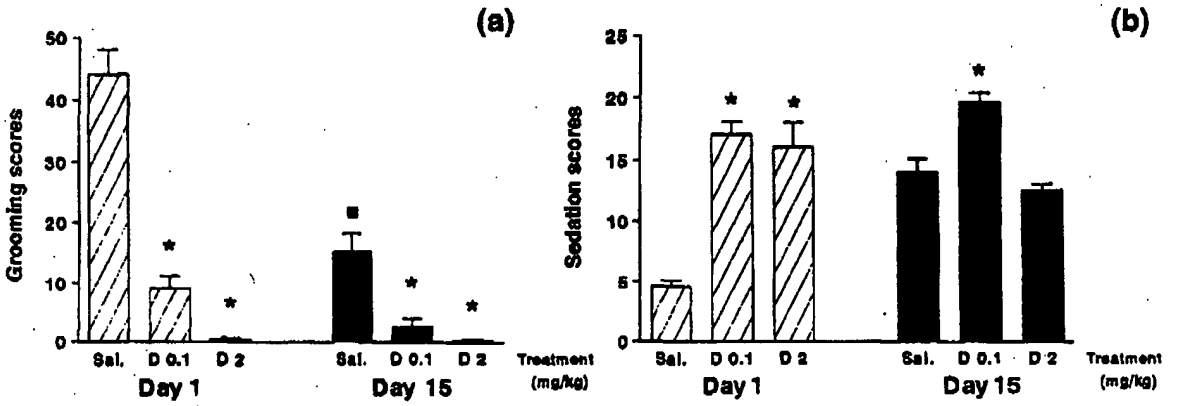

Figure 3 Effects of acute and chronic 7-OH-DPAT on (a) grooming and (b) sedation in male mice. Mice were treated with saline (Sal.) or 7-OH-DPAT (D) at 0.1 and 2 mg/kg 25 min before the test (50 min). Each histogram is the mean ± SEM of the scores attributed to each animal in the test period (six mice in each treatment group). *Significantly different from animals treated with saline on the same day (Kruskal–Wallis followed by Mann–Whitney U-test). ■ Significantly different from the same group of animals on Day 1 (Mann–Whitney U-test)

as they also increased motor activity (Borsini and Meli, 1988), this has now been ruled out for 7-OH-DPAT, as it produces hypolocomotion in the actimeter both at low and high doses. After chronic injection, 7-OH-DPAT abolished the immobility time in the tail-suspension test at 2 mg/kg; the same treatment did not modify the locomotor activity with respect to controls in the actimeter. This disjunction between effects in the tail-suspension test and locomotor activity was also indirectly confirmed in the experiment on grooming and sedation in acclimatized mice observed singly in cages. It should be pointed out that no stereotypical behaviour was recorded at the highest acute or chronic dose (data not reported). Given the uncertain selectivity of 7-OH-DPAT for D_2 or D_3 receptors, it is premature to speculate which receptors are involved in the effects produced in the tail-suspension test; moreover, most compounds that were formerly thought to be DA D_2 selective also bind to the DA D_3 receptors. However, our data support the putative involvement of dopaminergic mechanisms in mediating an antidepressive-like state in animals; this was achieved after acute and chronic, relatively high doses of the DA agonist 7-OH-DPAT in the tail-suspension test.

A number of intriguing reports merit consideration, as they are consistent not only with the hypothesis that DA depletion is involved in depressive states but also with our data reporting an 'antidepressant profile' for 7-OH-DPAT. First of all, dopaminergic mechanisms on the chronic mild stress model have been discussed extensively (Willner *et al.*, 1994). In particular, the importance of antidepressant sensitization of mesolimbic DA receptors has been demonstrated in several studies (Spyraki and Fibiger, 1981; Borsini *et al.*, 1985; Serra *et al.*, 1989) and antidepressant-like effects have been shown in stress-induced anhedonia for the D_2-D_3 agonist pramipexole (Willner *et al.*, 1994). Repeated treatments with antidepressants selectively increases DA transmission in limbic areas (Spyraki and Fibiger, 1981) and a growing number of laboratory experiments have demonstrated that chronically-administered antidepressants enhance behavioural stimulant responses to DA agonists.

In conclusion, in view of the activity of (-)eticlopride and 7-OH-DPAT on D_2/D_3 receptor subtypes, the effects displayed by the two compounds in the tail-suspension test support the dopaminergic modulation of a 'depressive-like state' in animals and suggest that further investigations on their potential usefulness as antidepressants are warranted.

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Neurobiology

Elevated anxiety and antidepressant-like responses in serotonin 5-HT_{1A} receptor mutant mice

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ABSTRACT **The brain serotonin (5-hydroxytryptamine; 5-HT) system is a powerful modulator of emotional processes and a target of medications used in the treatment of psychiatric disorders. To evaluate the contribution of serotonin 5-HT_{1A} receptors to the regulation of these processes, we have used gene-targeting technology to generate 5-HT_{1A} receptor-mutant mice. These animals lack functional 5-HT_{1A} receptors as indicated by receptor autoradiography and by resistance to the hypothermic effects of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Homozygous mutants display a consistent pattern of responses indicative of elevated anxiety levels in open-field, elevated-zero maze, and novel-object assays. Moreover, they exhibit antidepressant-like responses in a tail-suspension assay. These results indicate that the targeted disruption of the 5-HT_{1A} receptor gene leads to heritable perturbations in the serotonergic regulation of emotional state. 5-HT_{1A} receptor-null mutant mice have potential as a model for investigating mechanisms through which serotonergic systems modulate affective state and mediate the actions of psychiatric drugs.**

The brain serotonin (5-hydroxytryptamine; 5-HT) system has been strongly implicated in the neural regulation of mood and anxiety state. Accordingly, many commonly used antidepressant and antianxiety medications target this system (1). The complex physiological actions of serotonin are mediated by a heterogeneous family of at least 14 distinct receptor subtypes (2). Although the relative contributions of individual receptor subtypes to the serotonergic regulation of mood are incompletely understood, particular attention has focused on the 5-HT_{1A} receptor subtype. Partial agonists at this receptor, such as buspirone, are in clinical use as anxiolytics (3), and 5-HT_{1A} receptor antagonists are reported to accelerate the therapeutic effects of antidepressant medications (4).

These compounds produce complex effects on brain function through interactions with functionally distinct populations of 5-HT_{1A} receptors. 5-HT_{1A} receptors located on serotonergic neuronal cell bodies and dendrites are the predominant somatodendritic autoreceptors of these neurons; their activation suppresses serotonergic neuronal activity (5, 6). In addition, postsynaptic 5-HT_{1A} receptors are expressed in numerous serotonergic projection sites such as the cerebral cortex, septal nuclei, hippocampus, and amygdala (7). The relatively selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) and antagonist WAY 100635 (8) have been used as pharmacological probes of 5-HT_{1A} receptor function. Systemic administration of 8-OH-DPAT produces hyperphagia, hypothermia, and an anxiolytic-like effect in rodents (9–12). The behavioral and physiological effects of 8-OH-DPAT are blocked by pretreatment with WAY 100635 (12–14).

To complement pharmacological approaches to the study of 5-HT_{1A} receptor function, we have used a gene-targeting strategy to generate a line of mice bearing a complete and specific elimination of this receptor subtype. We report that these animals display a pattern of behavioral changes that sheds light on the functional roles of the 5-HT_{1A} receptor subtype in neural pathways relevant to anxiety and depression.

MATERIALS AND METHODS

Targeting Vector. An 80- to 100-kb genomic fragment was isolated from a 129/Sv genomic P1 library (Genome Systems, St. Louis) by using PCR primers generated to sequences within the intronless 5-HT_{1A} receptor gene protein-coding region. Gene fragments were subcloned into a pBluescript II SK (Stratagene) vector for further manipulation. A 1.5-kb *Pst*I genomic fragment, including a portion of protein-coding region, was replaced by a neomycin resistance cassette under the regulation of a phosphoglycerate kinase promoter (Fig. 1*A*). This mutation was designed to produce a loss of function by truncating the 5-HT_{1A} receptor protein at the third cytoplasmic loop. The neomycin resistance cassette was flanked by 1.7 kb of homologous genomic sequence at its 5′ aspect and by 6.9 kb of homologous genomic sequence at its 3′ aspect. The mutated fragment was cloned into a phosphoglycerate kinase–thymidine kinase plasmid (PGK-TK) containing the herpes simplex virus thymidine kinase gene driven by the phosphoglycerate kinase promoter in a Bluescript SK vector.

Generation of Homologous Recombinant Clones. 129/SvJ-derived JM1 embryonic stem (ES) cells (15) were electroporated with linearized targeting vector, and drug selection was applied in a positive/negative selection strategy (16) to enrich for targeted clones. Surviving ES cell colonies were screened for homologous recombination by using Southern blot analysis. A genomic fragment corresponding to a region 5′ to the expected integration site was used to probe genomic DNA digested with *Bam*HI. Wild-type and mutant alleles were indicated by 12.3-kb and 6.5-kb fragments, respectively (Fig. 1).

Generation of 5-HT_{1A} Receptor-Null Mutant Mice. Male chimeras produced by injection of targeted ES cells into C57BL/6J blastocysts were bred with C57BL/6J females. Germ-line transmission of the targeted mutation was verified by Southern blot analysis of tail DNA. Heterozygotes were then mated with C57BL/6J mice, and the resulting heterozygous animals were crossed, producing homozygous mutant,

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Abbreviations: 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; 5-HT, 5-hydroxytryptamine; ES, embryonic stem.

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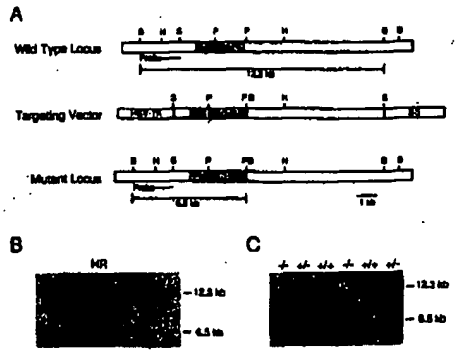

FIG. 1. (*A*) A schematic representation of the 5-HT_{1A} receptor gene, targeting construct, and the mutant allele. The region corresponding to the probe used for Southern blotting is indicated. (*B*) A representative Southern blot of *Bam*HI-digested ES cell genomic DNA; the wild-type allele is indicated by a 12.3-kb band and the homologous recombinant (HR) allele indicated by a 6.5-kb band. (*C*) Southern blot of mouse tail genomic DNA from wild type (+/+), heterozygous (+/−), and homozygous mutant (−/−) animals. HSV-TK, herpes simplex virus thymidine kinase gene; BS, pBluescript SK vector; NEO, neomycin resistance cassette; B, *Bam*HI; H, *Hin*dIII; S, *Sal*I; P, *Pst*I.

heterozygous, and wild-type mice. Animals of this generation were used for the studies reported. Mice were group-housed 2–6 mice per cage with free access to food and water under a 12-hr light/dark cycle (lights on at 0600 hr). For behavioral and physiological experiments, investigators were blind to animal genotype.

Northern Blot Analysis. Northern blot analysis was performed by using 20 μg of whole-brain total RNA extracted with the guanidinium isothiocyanate/phenol procedure (17). A 1.5-kb genomic fragment corresponding to the region deleted from the targeting vector was ^{32}P-radiolabeled by random prime labeling. After hybridization, washes, and film development, blots were stripped and rehybridized with a cyclophilin probe (internal standard).

Immunohistochemistry. Mice were perfused pericardially with PBS followed by a 4% paraformaldehyde solution. Brains were post-fixed in the same fixative, cryoprotected in sucrose solutions (in PBS), frozen in powdered dry ice, and stored in −70°C until use. Cryostat (40-μm) sections from comparable levels of wild-type and mutant brains were selected and processed in parallel. Sections were incubated for 1 hr at room temperature in a blocking solution followed by incubation with the primary antibody for 2 days at 4°C. Primary antibodies and their dilutions were rabbit anti-serotonin (Incstar, Stillwater, MN; 1:15,000 dilution), rabbit anti-serotonin transporter (Incstar, 1:12,500 dilution), rabbit anti-tyrosine hydroxylase (Pel-Freez Biologicals; 1:5,000 dilution). A biotinylated goat anti-rabbit secondary antibody (Vector Laboratories; 1:200 dilution) was applied for 30 min, followed by a 30-min incubation in avidin-biotin horseradish peroxidase complex (Vector Laboratories). Peroxidase label was detected by incubation in 3,3′-4,4′-diaminobenzidine (DAB; Sigma).

Receptor Autoradiography. Fresh frozen brains were cut into 20-μm sections and stored at −20°C until use. Immediately after thawing, sections were incubated for 15 min in incubation buffer (0.17 M Tris·HCl, pH 7.7/4 mM $CaCl_2$/0.01% ascorbic acid/10 μM pargyline). To assess nonspecific binding, one of each pair of adjacent sections was incubated for another 10 min in incubation buffer with 10 μM serotonin. Sections were then incubated with 2 nM [^{3}H]8-OH-DPAT (NEN) for 45 min, followed by two 5-min washes in ice-cold incubation buffer. Sections were then dried with a stream of cool air, apposed to Hyperfilm-^{3}H (Amersham), and developed with D-19 solution (Kodak) after a 2-wk exposure.

Tissue Monoamine Determinations. After decapitation, dissection of the medial prefrontal cortex, hippocampus, and hypothalamus were rapidly performed, and tissue samples were frozen and stored at −70°C until analysis. Monoamines and their metabolites were extracted from tissue homogenates by using perchloric acid and then quantified from 100-μl volumes of the supernatant injected onto an HPLC system: 4 × 300 mm column; 5 μM Resolve C_{18} stationary phase (Waters); mobile phase of 166 mM citric acid, 25 mM sodium acetate, 0.1 mM Na_2·EDTA, 1.2 mM sodium octyl sulfate, 28.9 mM triethylamine, and 10% MeOH (vol/vol; apparent pH 2.5). The flow rate was 0.5 ml/min. Analytes were detected electrochemically, and monoamine concentrations were estimated by using external calibration curves that were generated daily.

Behavioral Testing. Behavioral assays were performed in mice between the ages of 10 and 14 weeks. Approximately 3 min before each assay, animals were removed from their home cage and placed in a clean holding cage, unless otherwise specified. Between subjects, instruments were cleaned with a 0.25% bleach solution, wiped down with water, and then dried. All standard polycarbonate mouse (29 × 18.5 × 13 cm) and rat (48 × 27 × 13 cm) cages used during testing were autoclaved between subjects. Subjects were divided into two groups; one group was assessed in the home-cage activity, open-field, and elevated-zero maze tests (n = 10 per genotype), whereas the other was examined in the rotorod, tail-suspension, and thermoregulation assays (n = 9 per genotype). All subjects were included in the novel-object assay. Unless otherwise noted, all animals were tested in a particular behavioral assay on the same day during the light cycle. All experimental conditions were counterbalanced by genotype.

Home-Cage Activity. Animals were housed individually in rat cages with bedding, food, and water. To assess activity, beam breaks were collected each hr for 72 hr with a photobeam activity system (San Diego Instruments). Both horizontal locomotor activity (4 × 8 array of infrared photobeams) and rearings (elevated set of eight infrared photobeams) were monitored. The first 24 hr were considered an acclimation period, and the subsequent 48 hr of activity were analyzed.

Rotorod. Motor coordination was assessed with an Accurotor rotorod (Accuscan Instruments, Columbus, OH) set at an acceleration rate of 2 rpm per 15 sec. Four animals were tested concurrently in separated 11-cm-wide compartments on a rod approximately 3 cm in diameter and elevated 35 cm. Each animal was assessed 3 times with a 1-hr intertrial interval. Performances in trials 1–3 were averaged for data analysis.

Open Field. A 4-unit open field was used, consisting of a white Kydex box divided into 4 separate 50 × 50 × 38 cm chambers, allowing 4 animals to be tested concurrently. A video camera was mounted directly above the chambers. Each chamber was divided into peripheral (within 7 cm of the chamber walls) and central (area within the periphery) regions. Distance traveled, time spent in the central vs. peripheral areas of the field, and number of entrances into the central area were assessed for 30 min with a video tracking system (Poly-Track, San Diego Instruments).

Elevated Zero Maze. This maze is an elevated (42 cm), white, annular (46-cm diameter) runway (5.5-cm width) divided into 4 quadrants; 2 opposing "open" quadrants without walls (3-mm lip) and 2 opposing "closed" quadrants (11-cm high walls). A video camera was mounted directly above the maze. Mice were placed in the closed quadrant of the maze, and activity was collected for 5 min. Time spent in the open quadrants was monitored with a stopwatch. Numbers of open-quadrant entrances, head dips, stretch-attend postures, rearing, and fecal boli were also recorded. Total activity and activity in the open quadrants of the maze were recorded automatically by using the video tracking system. Animals received 2 test sessions, 2 weeks apart.

Novel Object. Approximately 16 hr before novel-object exposure, animals were housed individually in rat cages with bedding, food, and water, and activity was collected with the photobeam system. During the light cycle (1030–1230 hr), a novel object (white table tennis ball, Harvard, 2-Star) was taped to the corner of the cage farthest from the nest, and activity was monitored for the next 30 min. Beam breaks were collected to record activity and time and entrances into the area where the novel object was located (defined as a 175-cm^2 region bounded by the corner of the cage). Latency to enter this region was scored with a stopwatch. Time spent in the nest area while the novel object was in the cage was also determined (also defined as a 175-cm^2 region).

Tail Suspension. Mice were suspended by the tail from a metal bar (1.2-cm diameter) elevated 30 cm in a visually isolated area. Behavior was videotaped for 6 min. Immobilization time during tail suspension was scored with a stopwatch from the videotape. Animals were tested in 2 groups on consecutive days.

8-OH-DPAT-Induced Hypothermia. Mice were transferred to holding cages 30 min before drug administration. (+)-8-OH-DPAT (Research Biochemicals, Natick, MA) was dissolved in 0.9% saline and injected subcutaneously (0, 0.05, 0.2, and 1.0 mg/kg). A thermistor probe was inserted 1.5 cm into the rectum, and the temperature reading was recorded (TH-5 thermometer; Physitemp, Clifton, NJ) 20 and 10 min before and 10, 20, 30, 40, 50, and 60 min after drug treatment. Each animal received all doses in a counterbalanced manner, with a 4-day latency period between treatments.

Statistics. All behavioral scores were analyzed for normality by using the Shapiro-Wilk's W test. One-way ANOVA or repeated-measures ANOVA followed by Tukey HSD post hoc tests were used to compare the effect of genotype on normally distributed variables. Genotype comparisons of variables that were not normally distributed were analyzed with the Kruskal–Wallis H test followed by Tukey HSD post hoc tests. All figures display the mean ± SE of the data to illustrate the central tendency of the variables. For all analyses, significance was assigned at the $P \leq 0.05$ level.

RESULTS

Homologous recombinant ES cell clones were produced with an overall frequency of approximately 1/100 drug-resistant colonies (Fig. 1*B*). Blastocyst injections of targeted cells resulted in chimeric mice that were bred with C57BL/6J females. Germ-line transmission of the mutation was confirmed by Southern blot analysis (Fig. 1*C*). Heterozygote crossing produced wild-type, heterozygous, and homozygous mutant mice in the expected Mendelian ratios, indicating that the mutation does not impair embryonic viability. 5-HT_{1A} receptor mutant mice were healthy and normal in appearance.

To assess the abundance of intact 5-HT_{1A} receptor mRNA within the brains of mutant mice, Northern blot analysis was performed. The blot was probed with a radiolabeled fragment corresponding to the deleted region of the gene. A ≈5.5-kb band was apparent in the wild-type, but not the mutant, lane (Fig. 2*A*). A ≈50% reduction in the abundance of intact transcript was observed in heterozygous animals. To detect 5-HT_{1A} receptor-binding sites, receptor autoradiography was performed using [^{3}H]8-OH-DPAT (Fig. 2*B*). In wild-type sections, the distribution of binding conformed to prior reports (7), and no specific autoradiographic signal was observed in brain sections from homozygous mutant mice. Heterozygous sections exhibited an intermediate level of binding.

No differences were observed in brain weights of wild-type, heterozygous, or homozygous mutant mice. Evaluation of Nissl-stained sections throughout the neuraxis revealed no apparent cytoarchitectural abnormalities. Serotonin immunocytochemistry revealed no overt abnormalities in the distribution of serotonergic neurons (Fig. 3) in homozygous mutant mice. Similarly, serotonin transporter immunocytochemistry revealed no apparent differences in the distribution or density of serotonergic fiber staining. For example, the distribution of serotonergic fibers within the hippocampal formation appeared normal in mutant brains, with the characteristic dense innervation of the lacunosum moleculare layer of Ammon's horn (Fig. 3). Because of known interactions between serotonin and catecholaminergic neural systems, the distribution of catecholaminergic neurons was assessed by using tyrosine hydroxylase immunocytochemistry; no phenotypic differences were noted (Fig. 3). Levels of serotonin, dopamine, and their metabolites were also determined in mutant mice. HPLC analysis of extracts from the prefrontal cortex, hippocampus, and hypothalamus revealed no abnormalities (data not shown).

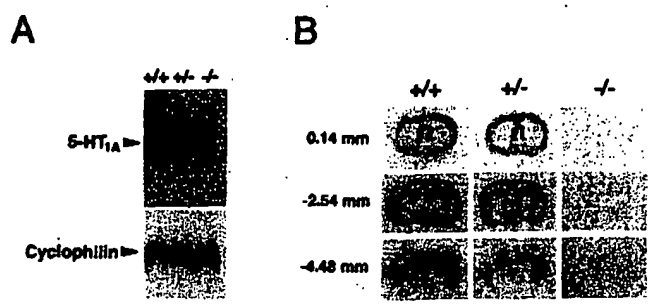

FIG. 2. (*A*) Northern blot analysis of 5-HT_{1A} receptor gene expression in wild-type (+/+), heterozygous (+/−), and homozygous mutant (−/−) animals. A cyclophilin probe demonstrated equivalent RNA loading in all lanes. (*B*) [^{3}H]8-OH-DPAT autoradiography of representative sections at the level of the septal nuclei (*Top*), mid-hippocampal formation (*Middle*), and dorsal raphe nucleus (*Bottom*). Anterior/posterior coordinates in mm relative to bregma are indicated, in accordance with the mouse-brain atlas of Franklin and Paxinos (39).

Diurnal patterns of activity were monitored for animals individually housed in photobeam enclosures. A repeated-measures ANOVA revealed a significant main effect of time on activity, such that animals were more active in the dark

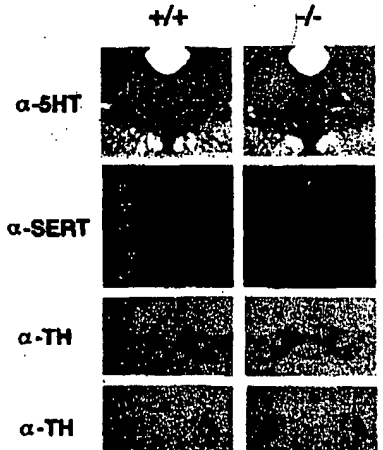

FIG. 3. Immunohistochemical analysis of monoaminergic neurons in wild-type (+/+) and homozygous mutant (−/−) brains. Dorsal raphe serotonin neurons labeled with an anti-serotonin antibody (α-5-HT). Darkfield view of serotonergic innervation of the dorsal hippocampus labeled with an anti-serotonin transporter antibody (α-SERT). Dopaminergic neurons of the substantia nigra (SN) and ventral tegmental area (VTA) labeled with an anti-tyrosine hydroxylase antibody (α-TH). Noradrenergic neurons of the locus coeruleus (LC) labeled with α-TH. PY, CA1 pyramidal layer; SR, striata radiatum; LM, lacunosum moleculare; DM, dentate gyrus molecular layer; GR, dentate gyrus granule-cell layer.

phase than in the light phase ($F_{1,29}$ = 30.21, $P \leq 0.01$). However, no phenotypic differences in activity levels or diurnal activity patterns were observed [48-hr total distance (cm) during light cycle: +/+, 10,933 ± 830, +/−, 15,220 ± 2,525, −/− 13,170 ± 2,428; dark cycle: +/+, 47,733 ± 6,370, +/−, 39,173 ± 6,095, −/−, 36,058 ± 3,518]. Similarly, one-way ANOVA revealed no differences by genotype in motor coordination on the accelerating rotorod assay.

Analysis of open-field behavior revealed a significant effect of genotype on time spent ($F_{2,25}$ = 6.65, $P \leq 0.01$), distance traveled ($F_{2,25}$ = 10.20, $P \leq 0.01$), and entrances into the central area of the enclosure ($F_{2,25}$ = 12.09, $P \leq 0.001$; Fig. 4). Post hoc comparisons indicated that wild-type mice spent more time, were more active, and entered the center of the open field more frequently than did mutant and heterozygous animals. No differences in total activity in the open field by genotype were observed.

Significant phenotypic effects were found in open-quadrant behavior in the elevated-zero maze. As these effects did not differ between trials 1 and 2, data were collapsed for analysis (Fig. 5). A significant genotype effect was found on time spent ($F_{2,26}$ = 9.28, $P \leq 0.01$) and distance traveled (Kruskal–Wallis H_2 = 6.17, $P \leq 0.05$) in the open quadrants (Fig. 5 *A* and *B*). Post hoc comparisons indicated that open-quadrant time and activity were reduced in mutant mice relative to wild-type animals. Mutants also displayed less time in the open quadrants than did heterozygotes. ANOVA revealed a trend toward a phenotype effect in open-quadrant entrances; direct comparisons of mutant and wild-type groups revealed a significant difference (t_{17} = 2.44, $P \leq 0.05$; Fig. 5*C*), indicating that mutants were less likely to enter the open quadrants. A significant effect was also observed in the frequency of head dips displayed by genotype ($F_{2,28}$ = 4.35, $P \leq 0.05$; Fig. 5*D*), with heterozygous animals exhibiting a greater number than mutant mice. No differences in total activity or frequency of stretch-attend postures, fecal boli, or rearing were observed by genotype on the elevated-zero maze.

Marked differences also were found in the response of mutant and wild-type animals to the presentation of a novel object. A significant genotype effect was observed in the latency to approach the object (H_2 = 9.95, $P \leq 0.01$; Fig. 6*A*), with homozygous mutants exhibiting significantly greater latencies than heterozygous or wild-type mice. Significant phenotypic differences also were found in the number of entries

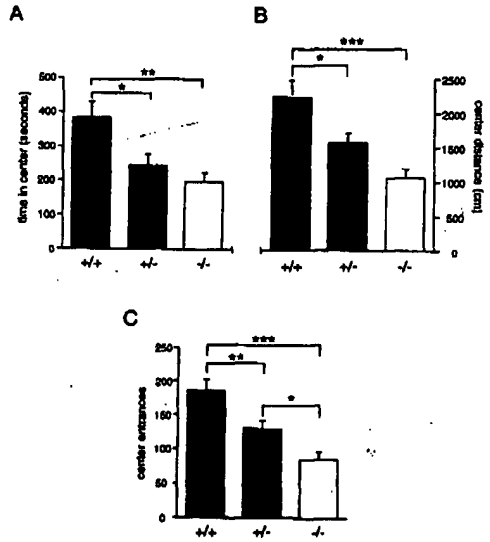

FIG. 4. Locomotor behavior in the open field. Mean (±SEM) time (*A*), distance (*B*), and entrances (*C*) into the central region of an open field during a 30-min exposure. Significant differences by genotype are indicated as ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$.

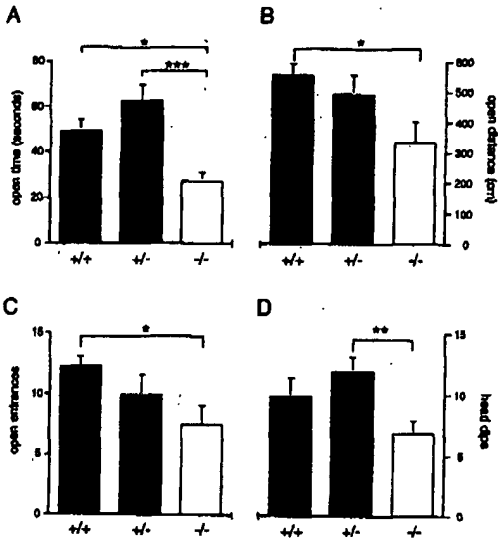

FIG. 5. Behavior in the elevated-zero maze. Mean (±SEM) time (*A*), distance (*B*), open-quadrant entrances (*C*) and head dips (*D*) in the open quadrants of an elevated-zero maze during a 5-min trial. Significant differences by genotype are indicated as ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$.

into the region of the novel object (H_2 = 7.40, $P \leq 0.05$; Fig. 6*B*) and time spent in the nest area (H_2 = 6.93, $P \leq 0.05$) during the 30-min test session. Post hoc analysis indicated that mutant mice were less likely to approach the novel object and more likely to spend time in their nest than wild-type mice. Although no differences in overall activity by genotype were evident before novel-object presentation, a significant genotype effect was observed in horizontal (H_2 = 9.22, $P \leq 0.01$) and vertical (H_2 = 10.83, $P \leq 0.01$) activity in the presence of the novel object (Fig. 6 *C* and *D*). Post hoc analysis showed that wild-type mice exhibited greater horizontal and rearing activity than mutant mice. Heterozygous animals also displayed more rearing than mutant mice.

A substantial effect of genotype was also observed on the tail suspension test ($F_{2,20}$ = 26.49, $P \leq 0.001$; Fig. 7). Post hoc analyses indicated that wild-type mice spent significantly more

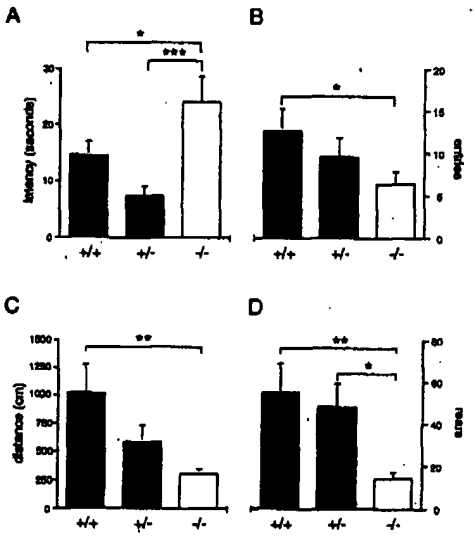

FIG. 6. Responses to a novel object. Mean (±SEM) (*A*) latency to approach a novel object placed in a familiar environment; (*B*) number of entries into area containing a novel object during a 30 min trial; (*C*) horizontal and (*D*) vertical activity during 30-min novel-object exposure. Significant differences by genotype are indicated as ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$.

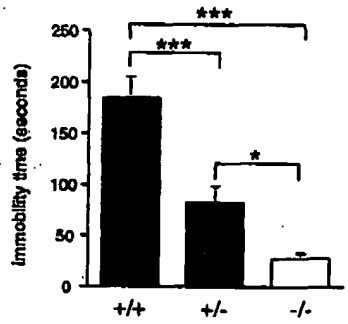

FIG. 7. Immobility in the tail-suspension assay. Mean (±SEM) time immobilized during the 6-min test. Significant differences by genotype are indicated as ***, $P \leq 0.001$; *, $P \leq 0.05$.

time immobile when suspended by the tail than did mutant and heterozygous animals, and that heterozygotes spent more time immobile than did homozygous mutant mice. Mutants displayed an 85% reduction in immobility relative to wild-type mice.

The above results were obtained in male mice. Female mice were separately assessed in the open-field, elevated-zero maze, and tail-suspension assays ($n = 10$ per genotype). Phenotype differences among females were less pronounced, but similar to males, with significantly elevated anxiety-like behavior in the open field and elevated-zero maze and reduced immobility in the tail-suspension assay in the mutants (data not shown).

To confirm the absence of 5-HT_{1A} receptor-mediated physiological responses in homozygous 5-HT_{1A} receptor mutant mice, thermoregulatory responses to 8-OH-DPAT were assessed. In mice, the hypothermic effects of this compound have been proposed to reflect its actions at somatodendritic 5-HT_{1A} autoreceptors (18). Because this effect peaked at 20 min posttreatment, changes in body temperature from baseline to this time were used for analysis. Repeated-measures ANOVA revealed a significant main effect of treatment ($F_{3,66} = 73.01$, $P \leq 0.001$) and genotype ($F_{2,22} = 29.32$, $P \leq 0.001$), and an interaction between treatment and genotype ($F_{6,66} = 13.24$, $P \leq 0.001$; Fig. 8). Post hoc comparisons indicated that the higher doses of 8-OH-DPAT (0.2, 1.0 mg/kg) were associated with hypothermia in wild-type and heterozygous animals. However, 5-HT_{1A} receptor mutant mice were insensitive to the hypothermic effects of 8-OH-DPAT at all doses administered.

DISCUSSION

We report the generation and behavioral analysis of a mouse strain bearing a targeted disruption of the serotonin 5-HT_{1A} receptor. The absence of functional 5-HT_{1A} receptors in these animals was confirmed by Northern blot analysis and receptor autoradiography and by the insensitivity of homozygous mutants to the hypothermic effect of 8-OH-DPAT. In accord with a substantial contribution of this receptor subtype to the serotonergic regulation of behavior, we find evidence for enhanced anxiety in the homozygous mutants and a marked antidepressant-like response in a rodent depression model. These behavioral phenotypes are not readily accounted for by perturbations of brain development. Examination of brain weights and Nissl-stained sections revealed no overt morphological abnormalities in 5-HT_{1A} receptor mutant mice. In addition, no abnormalities of serotonin system development were indicated by immunocytochemical analysis of serotonergic cell bodies and fibers or by determination of brain serotonin and 5-hydroxyindoleacetic acid (5-HIAA) content. These results are notable in light of the proposal that glial 5-HT_{1A} receptors influence brain development by stimulating the release of the neurotrophic factor S-100β, a protein that modulates the differentiation of neocortical and serotonergic neurons (19). These results do not exclude such a role for the 5-HT_{1A} receptor, as compensatory mechanisms may minimize the impact of its absence. In addition, the possibility that subtle developmental abnormalities exist cannot be excluded.

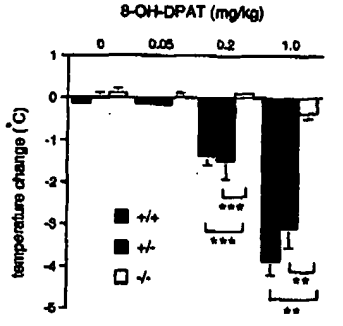

FIG. 8. Thermoregulatory responses to the administration of 8-OH-DPAT. Mean (±SEM) reduction in body temperature 20 min posttreatment (vehicle or 0.05, 0.2, 1.0 mg/kg 8-OH-DPAT; SC). Significant differences are indicated as ***, $P \leq 0.001$; **, $P \leq 0.01$.

Homozygous mutant mice displayed consistent elevations of anxiety-related behaviors in several assays that could not be accounted for by generalized phenotypic differences in locomotor activity. In the open field, mutants displayed a significant reduction in exploration of the central area, remaining in close proximity to the walls of the enclosure. The aversion to open spaces and seeking of cover, known as thigmotaxis, is a rodent behavior believed to correlate with anxiety (20, 21). Thigmotaxic responses were also assessed in the elevated-zero maze assay, a pharmacologically validated tool for the assessment of anxiety that measures aversion to an elevated open platform (22). Consistent with an increase in anxiety, mutants exhibited reduced time and activity in the open quadrants of the maze. In addition, they displayed fewer head dips, an exploratory behavior believed to inversely correlate with anxiety state (22). Moreover, mutants exhibited increased avoidance of a novel object, as indicated by elevated latencies to approach the object, diminished frequency of approach, and increased time spent in the nest area. Novel-object avoidance is believed to correlate with anxiety state (23). Thus, increased anxiety-like behaviors in response to an aversive environmental context generalized to a discrete novel object. Together, these findings suggest that 5-HT_{1A} receptor mutant mice exhibit elevated levels of anxiety.

Systemic administration of 5-HT_{1A} receptor agonists such as buspirone and antagonists such as WAY 100635 produce inconsistent effects on anxiety-related behaviors in various rodent models of anxiety (14, 24–27). This may relate to the existence of distinct 5-HT_{1A} receptor populations that produce opposing effects on anxiety regulation. For example, the stimulation of postsynaptic 5-HT_{1A} receptors of the dorsal hippocampus and amygdala has been proposed to elicit anxiogenic effects (28). In contrast, activation of 5-HT_{1A} autoreceptors is believed to produce anxiolytic effects via the suppression of serotonergic neuronal activity, with consequent decreased serotonin release in limbic terminal fields (6, 29–32). It is therefore possible that the enhancement of anxiety in 5-HT_{1A} receptor mutant mice reflects a disinhibition of serotonergic neuronal activity. Increased serotonin release in limbic terminal fields may enhance anxiety through the activation of other serotonin receptor subtypes. This possibility is not precluded by the normal tissue content of serotonin and 5-HIAA in the mutants, because the storage pools of serotonin vastly exceed extracellular serotonin content.

The modulation of serotonergic neural transmission is also proposed to be central to the therapeutic effects of antidepressant drugs, many of which increase the availability of serotonin at postsynaptic sites (33). It is therefore noteworthy that 5-HT_{1A} receptor mutant mice exhibited an 85% reduction

in immobility in the tail-suspension test, an assay used to evaluate the potential antidepressant efficacy of drugs. Antidepressants, including selective serotonin reuptake inhibitors, reduce the immobility displayed by mice following unsuccessful attempts to escape when suspended by the tail (34–36). The reduction in immobility time exhibited by 5-HT_{1A} receptor mutants is therefore indicative of a substantial antidepressant-like response.

5-HT_{1A} receptor activity is believed to influence the antidepressant effects of selective serotonin reuptake inhibitors such as fluoxetine. In the short term, the administration of such agents reduces serotonergic neuronal activity caused by the stimulation of 5-HT_{1A} autoreceptors. With chronic administration, however, these receptors are believed to gradually desensitize, leading to recovery of serotonergic neuronal activity; a phenomenon proposed to underlie the delayed onset of antidepressant action with these compounds (37). In accord with these phenomena, open-label studies have been conducted in which coadministration of 5-HT_{1A} receptor antagonists appears to accelerate the antidepressant effects of selective serotonin reuptake inhibitors (38). We therefore propose that the antidepressant-like responses of 5-HT_{1A} receptor mutants reflect a disinhibiton of serotonergic neuronal activity resulting from the absence of 5-HT_{1A} autoreceptors. Alternative explanations involving compensatory alterations of other neurotransmitter systems remain a possibility.

5-HT_{1A} receptor mutant mice provide a useful model for exploring the functional roles of this receptor subtype. Electrophysiological and microdialysis studies of these animals will aid in determining the extent to which their behavioral profile reflects enhanced serotonergic system activity. Evaluation of the neural mechanisms underlying the behavioral phenotypes of 5-HT_{1A} receptor mutant mice will shed light on the neural pathways relevant to the serotonergic regulation of anxiety and depression and on the actions of psychiatric drugs for these disorders.

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The Tail Suspension Test for Screening Antidepressant Drugs: Comparison of Movement in ICR and NMRI Mice

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Introduction

Recently Steru *et al.*[1] have proposed an innovative method for screening antidepressant drugs, called "tail suspension test (TST)." In this test a mouse is suspended by the tail and the mouse's movement is automatically measured and recorded. Antidepressants were shown to quite selectively activate the movement in the mice. Later, Heyden *et al.*[2] demonstrated that in this test, only the NMRI strain, not the ICR strain, responded differently to various drugs. Based on these observations, they suggested that the mouse strain used for the test is of crucial importance. In the present study, we analyzed the movements of both strains in more detail and compared the effect of two antidepressants on the movement in each mouse strain in order to investigate the cause of such differences in motility between the strains.

Materials and Methods

Naive male ICR (Charles River, Japan) and NMRI (Charles River, FRG) mice weighing 20–30 g were used. They were housed in groups of 20 mice each in plastic cages at room temperature of about 22°C, and had free access to food and water. Testing was performed during the light phase of the diurnal cycle. The test procedure was similar to that described by Steru *et al.* (1985), with modifications of the movement detector, recording device and method of data analysis. In the present study, the mouse was suspended by the tail from a hook connected to a spring 30 min after injection with desipramine (2–10 mg/kg), imipramine (10 mg/kg) or saline control. The mouse's movements were quantified electrically by contactless microangle potentiometers; With this technique, the electric signal is converted into a digital signal, recorded, and analyzed by a computer. The data are expressed in terms of as resting time (sec) and motility (g × 0.1 sec). A statistical analysis of the results was by Wilcoxon's test.

Results and Discussion

Because some signals were recorded from the mice even when they appeared to have ceased moving, the criteria by which immobility is defined must be precisely established. These signals may be artifacts arising from the respiration, heart beat, tremor of the mice and rebound of the spring rather than from body motions. Therefore, a mathematical "filter" was added to computer processing in order to remove artifact values. In filtering, when the filter value is low, signals from finer motions are included in the analysis. Fig. 1 shows changes in motility and resting time on 10 minutes TST of ICR and NMRI mice as a function of the filter value. As shown in this figure, ICR mice tended to be more active than NMRI mice at all filter values tested except at the extreme value of 50%.

Desipramine at doses from 2 to 10 mg/kg significantly increased motility in both the ICR

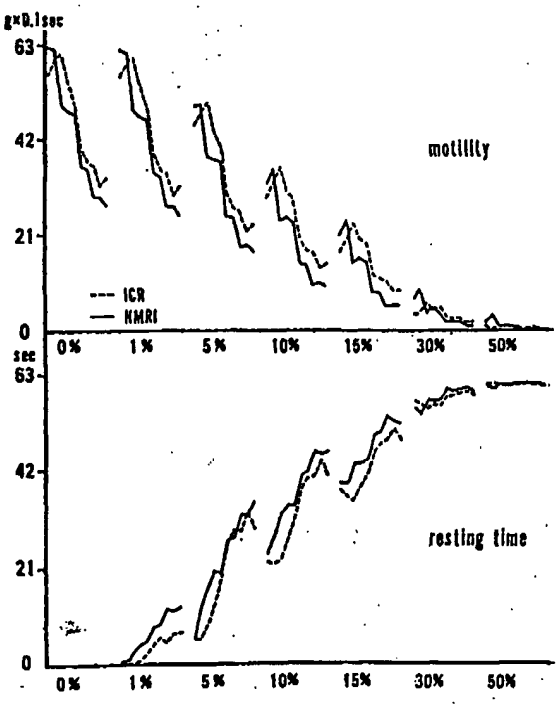

Fig. 1: Changes in motility and resting time on 10-minute tail suspension test (TST) of ICR and NMRI mice as a function of filter value.

and NMRI strains, while imipramine at doses of 10 mg/kg significantly increased the motility in the NMRI mice, but not in the ICR mice, as Heyden *et al.*[2] showed. These relative drug effects on each strain did not change with changes in the filter value. These results suggest that the different effects of the antidepressants on each strain were not due to differences in their effect on the motion of the strains, but to an intrinsic difference between the strains in overall activity level. However, there may be pharmacokinetic or pharmacodynamic differences between the strains, and a further study is necessary to clarify this issue.

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Use of the Automated Tail Suspension Test for the Primary Screening of Psychotropic Agents

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Abstract—Mice, when suspended by the tail, will alternate between active attempts to escape and immobility. A specially developed computerized device (ITEMATIC-TST) automatically measures the duration of immobility of 6 mice at one time and at the same time provides a measure of the energy expended by each animal, the power of the movements. Use of these 2 parameters enables activity profiles to be generated which can distinguish different classes of psychotropic activity. Immobility is decreased by antidepressants and psychostimulants, but increased by neuroleptics and minor tranquillizers. Minor tranquillizers can be distinguished from neuroleptics in that they decrease the power of the movements, whereas neuroleptics are without effect on this parameter. The present experiments were undertaken to see whether the activity profiles generated in this procedure could indeed be useful for primary psychotropic screening. Eighteen compounds, including antidepressants, neuroleptics, minor tranquillizers, sedative/hypnotics, dopaminergic stimulants and 3 dummy compounds were first submitted to a shortened primary observation procedure for dose finding and were then investigated at 2 doses in the automated tail suspension test. All experiments were conducted blind. The results obtained largely confirm the activity profiles already reported in this test and show that the combined use of primary observation and the automated tail suspension test permit the unambiguous identification of the pharmacological activity of 15 of the compounds tested with tentative identification of the 3 remaining compounds.

Introduction

Four essential requirements for a primary screening test for psychotropics are rapidity, sensitivity, reliability and objectivity (Turner, 1965). Rapidity is required to ensure that a maximum number of molecules can be screened in a minimum of time while maximising the output of individual laboratory technicians. Sensitivity refers to the necessity that a screening procedure does not miss important biological activity (false negatives). Reliability and objectivity are necessary to ensure that results can be repeated when required and do not depend overly on the subjective evaluation of the individual observer.

We have recently described a new behavioural testing procedure in mice, the tail suspension test (Stéru *et al.*, 1985a) and its computerized testing device, the ITEMATIC-TST (Stéru *et al.*, 1985b, 1986; Porsolt *et al.*, 1986). This test was first developed as a procedure for detecting antidepressant activity and was inspired from the "behavioural despair" test described by one of us several years ago (Porsolt *et al.*, 1977). Both tests are based on the principle that animals, exposed to an aversive situation from which there is no escape, will, after periods of vigorous activity, cease attempts to escape and become immobile. Immobility in both procedures is reduced by a wide variety of antidepressants.

The computerized tail suspension procedure (ITEMATIC-TST), in addition to measuring immobility, also measures the power of the movements. Preliminary findings with these 2 parameters (Stéru *et al.*, 1985b, 1986; Porsolt *et al.*, 1986) suggested that the automated procedure was capable of recognizing different classes of psychotropic activity. For example, antidepressants and psychostimulants decreased immobility and at the same time either increased or had no effect on the power of the movements depending on the compound. Neuroleptics and minor tranquillizers, on the other hand, increased immobility but differed in that neuroleptics had little effect on the power of the movements, whereas this parameter was clearly decreased by benzodiazepines.

These findings suggest that different classes of psychotropics have different and recognizable profiles of activity in the automated tail suspension procedure and that this procedure could thus serve as a primary screening test satisfying the criteria enumerated above.

The experiments reported in the present paper were aimed to investigate this possibility by testing a large number of different compounds under blind conditions in a simulated real life screening situation. As would be the case in the screening of totally unknown compounds, all substances were first subjected to a standardized dose finding procedure which consisted of a preliminary toxicity test coupled with a shortened primary observation test (Irwin, 1968), in the morning, followed in the afternoon by

the tail suspension test. The results obtained suggest that the combination of the 2 behavioural procedures (primary observation and the tail suspension test) permit the rapid and correct identification of the pharmacological activity of most of the compounds investigated.

Methods

Experimental animals and general experimental conditions

The animals used in this study were male NMRI mice, weighing 20–25 g, obtained from CERJ (Le Genest-St-Isle, Mayenne, France). The mice were delivered to the laboratory at least 3 days before the experiments and were housed in groups of 10 in macrolon cages (25.5 × 19.5 × 13.5 cm) with free access to food and water. The housing facility was maintained on a non-inverted artificial light/dark cycle with the light turned on at 8:00 and extinguished at 20:00. The ambient temperature within the animal house and the laboratories was maintained between 20 and 22° C.

Drugs

All compounds were delivered to the laboratory in number coded identical brown glass bottles containing a fixed quantity (1 g) in powder form. To ensure a maximum of anonymity, the numbers on the bottles were randomized as regards the compounds they contained and the tests were also conducted in a randomized order. The codes were broken only after the experiments were completed and the observations evaluated.

The following compounds were investigated: (+)-amphetamine sulphate (Coopération Pharmaceutique Française, batch C 11938), apomorphine hydrochloride (Coopération Pharmaceutique Française, batch C 11292), chlorpromazine hydrochloride (Specia, batch 5441 287), chlordiazepoxide (Hoffmann-La Roche, batch BA 150269), clorgyline hydrochloride (May and Baker, batch MB 9302/S1), desipramine hydrochloride (Ciba-Geigy, batch 505), diazepam (Hoffman-La Roche, batch 0306019), haloperidol (Janssen-Le Brun, batch B 15/3), imipramine hydrochloride (Ciba-Geigy, batch 519), nialamide hydrochloride (Pfizer, batch 51664), sodium barbital (Coger, batch GE 80051/33456), sodium pentobarbital (Coger, batch 10 967/D), pimozide (Janssen-Le Brun, batch B 12/1), sulpiride (Delagrange, ref. JB), viloxazine hydrochloride (ICI, batch ICI 58834). Three inert compounds were used as dummies: glucose-D (+) (Prolabo, batch 80108), sodium bicarbonate (Prolabo, batch 27778), sodium chloride (Prolabo, batch 85246).

Compounds were first tested for solubility. Soluble compounds were dis-

solved in distilled water. Insoluble compounds were dispersed in an acqueous suspension of 5 % arabic gum diluted qsp with distilled water.

All compounds were administered i.p. in a volume of 0.25 ml/20 g body weight. Doses are expressed in terms of the salt or base where appropriate.

Preliminary toxicity and behavioural observation (dose finding)

All compounds were submitted to a standardized dose finding procedure with the aim of determining 2 doses for investigation in the tail suspension test, the high dose being the first dose without lethality or important signs (e.g. hypothermia, loss of righting reflex) and the low dose being 1/4 of the high dose. Three mice were injected i.p. at 9:00 with a standard initial dose of 256 mg/kg and observed for lethality and behavioural signs 15, 30 and 60 min after injection. The behavioural signs were scored on an observation grid similar to that described by Irwin (1968) and contained the following items: sedation, excitation, aggressiveness, Straub tail, convulsions, tremor, exophtalmos, salivation, lacrimation, pilo-erection, defecation, fear, traction, reactivity to touch, righting reflex, sleep, motor incoordination, muscle tone, stereotypies, catalepsy, grasping, ptosis, respiration, corneal reflex, reaction to pain, gait. In addition, rectal temperature and pupil diameter were measured at each observation time.

A control group of 3 mice was always observed with each treated group and was injected i.p. with distilled water.

If no deaths or important signs (e.g. hypothermia, loss of righting reflex) were observed at 256 mg/kg of the test compound, the doses chosen for investigation in the tail suspension test were 32 and 128 mg/kg. If deaths were observed at 256 mg/kg, 32 mg/kg (dose reduction 8:1) was administered to another group of 3 animals and the observations were repeated. This general dose-finding procedure was continued until a first dose without lethality or important signs could be determined. In general, one technician could conduct a complete dose-finding study for 3 test compounds within the same morning session.

Tail Suspension Test

The procedure used followed that described by Stéru *et al.* (1986). Different groups of 10 mice were injected i.p. with 1 of the 2 doses of the test compound selected as described above and 30 min after injection were suspended by the tail in the ITEMATIC-TST apparatus (Steru *et al.*, 1986). The total duration of immobility and the power of the movements were measured during a 6 min test. With each compound tested 2 further groups were employed, a control group which received an i.p. injection of distilled water and a positive control group which received an i.p. injection of a standard dose of desipramine (32 mg/kg).

The order of testing different animals and the suspension units to which they were assigned were randomized according to a special programme generated by the ITEMATIC-TST. Testing was always conducted in the afternoon between 14:00 and 17:30. In general 3 test compounds were studied at 2 doses each in a comparison with the same control and positive control group. The 3 test compounds studied on any one afternoon were generally those for which the dose finding studies were conducted on the morning of the same day.

To ensure the reliability of the results 2 exclusion criteria were used; the mean immobility scores for the control group had to be greater than 55 sec and the immobility scores of the positive control group (desipramine 32 mg/kg) had to be significantly lower than those of the control group. Statistical significance was assessed using the Student's *t*-test for independent samples.

Results

The results obtained are presented in the following sections grouped according to substance class. It will be recalled that these experiments were conducted blind in a randomized order with the aim of seeing whether the procedures employed were capable of detecting different classes of psychotropic activity. We consider it of interest therefore to present not only the results themselves but also the conclusions drawn by the pharmacologist who directed the experiment (A. L.) before the different treatments were decoded. These conclusions are presented verbatim at the end of each section.

Effects of tricyclic and atypical antidepressants

The results obtained with desipramine, imipramine and viloxazine are shown in Table I. All 3 compounds showed toxicity at 256 mg/kg. At 32 mg/kg, the 3 compounds caused marked sedation with decreases in traction, muscle tone and reactivity to touch. Clear mydriasis was present with imipramine and desipramine and hypothermia was observed with desipramine and viloxazine. All 3 compounds clearly reduced the duration of immobility with most marked effects at the higher dose (32 mg/kg). There were no clear effects on the power of movements apart from a decrease at the high dose of imipramine.

Pharmacologist's conclusion

Desipramine: "The decrease in immobility observed during the tail suspension test together with the sedative and anticholinergic signs observed

during the primary observation test suggest a tricyclic-type antidepressant action."

Imipramine: "The decrease in immobility observed during the tail suspension test together with the sedative and anticholinergic signs in the

TABLE I

Effects of classical and atypical antidepressants in the rapid screening procedure

Test Compound: Desipramine			
		Primary Observation Test	
Dose mg/kg i.p.	Mortality	Behavioural change	Other signs
256	2/3	sedation decreased traction decreased muscle tone	ptosis hypothermia mydriasis
32	0/3	weak sedation	ptosis hypothermia mydriasis
		Tail Suspension Test	
		Immobility (% change from control)	Power of movement (% change from control)
32		−74***	+5
8		−11	+2

Test Compound: Imipramine			
		Primary Observation Test	
Dose mg/kg i.p.	Mortality	Behavioural change	Other signs
256	3/3	−	−
32	0/3	sedation decreased fear decreased muscle tone decreased reactivity	Marked mydriasis
		Tail Suspension Test	
		Immobility (% change from control)	Power of movement (% change from control)
32		−67**	−37**
8		−62*	−1

Table continued

TABLE I (*Continued*)

Test Compound: Viloxazine			
		Primary Observation Test	
Dose mg/kg i.p.	Mortality	Behavioural change	Other signs
256	3/3	–	–
32	0/3	sedation decreased fear decreased muscle tone decreased reactivity	hypothermia

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
32	−62**	+ 2
8	−52	+13

Student's *t*-test: * $p < 0.05$; ** $p < 0.01$; $p < 0.001$.

primary observation test suggest a tricyclic-type antidepressant action. The decrease in the power of the movements at 32 mg/kg suggests a myorelaxant component."

Viloxazine: "The decrease in immobility observed during the tail suspension test together with the sedative signs in the primary observation test suggest a tricyclic-type antidepressant action without an anticholinergic component."

Effects of monoamine oxidase inhibitors

The results obtained with clorgyline and nialamide are shown in Table II.

Neither clorgyline nor nialamide was toxic at the highest dose tested (256 mg/g). At 256 mg/kg, both compounds caused marked sedation with decreased muscle tone, decreased reactivity to touch, ptosis and hypothermia. Mydriasis was observed with clorgyline, but not with nialamide. Both compounds tended to decrease the duration of immobility in the tail suspension test but this effect was far from statistical significance. The power of movements was not affected.

Pharmacologist's conclusion

Clorgyline: "The phenomena observed during the primary observation together with the slight decreases in immobility in the tail suspension test

suggest that the compound is not devoid of psychopharmacological activity. Further tests would be required to identify this activity."

Nialamide: "The phenomena observed during the primary observation test together with the slight decrease in immobility during the tail suspension test suggest that further tests would be required to specify the psychotropic activity of the compound."

TABLE II

Effects of monoamine inhibitors in the rapid screening procedure

Test Compound: Clorgyline

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	0/3	marked sedation decreased reactivity decreased muscle tone	ptosis marked hypothermia mydriasis
32	0/3	slight sedation	hypothermia

Tail Suspension Test

	Immobility (% change from control)	Power of movement (% change from control)
32	−28	+4
8	−31	−7

Test Compound: Nialamide

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	0/3	moderate sedation decreased muscle tone decreased reactivity	ptosis hypothermia
64	0/3	–	slight ptosis

Tail Suspension Test

	Immobility (% change from control)	Power of movement (% change from control)
128	−24	+1
32	−19	+9

TABLE III

Effects of neuroleptics in the rapid screening procedure

Test Compound: Chlorpromazine

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	1/3	–	convulsions
32	0/3	marked sedation decreased fear decreased traction decreased reactivity decreased muscle tone	ptosis marked hypothermia
4	0/3	marked sedation decreased fear decreased reactivity decreased muscle tone	ptosis marked hypothermia myosis

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
1	+42	−16
0.25	+15	0

Test Compound: Haloperidol

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	0/3	marked sedation decreased fear decreased traction decreased reactivity decreased muscle tone catalepsy	ptosis abolition of corneal reflex marked hypothermia myosis
32	0/3	marked sedation decreased fear decreased traction decreased reactivity decreased muscle tone catalepsy 2/3	ptosis hypothermia myosis
4	0/3	moderate sedation	ptosis weak myosis

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
4	+128***	−35
1	+108**	−13

Table continued

TABLE III (*Continued*)

Test Compound: Pimozide			
		Primary Observation Test	
Dose mg/kg i.p.	Mortality	Behavioural change	Other signs
256	0/3	marked sedation decreased fear decreased reactivity decreased muscle tone	myosis
64	0/3	sedation	–

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
64	+13	−16
16	−25	+ 8

Test Compound: Sulpiride			
		Primary Observation Test	
Dose mg/kg i.p.	Mortality	Behavioural change	Other signs
256	0/3	–	slight ptosis

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
128	+71*	+1
32	+ 7	0

Student's *t*-test: * $p < 0.05$; *** $p < 0.001$.

Effects of neuroleptics

The effects of chlorpromazine, haloperidol, pimozide and sulpiride are shown in Table III.

Apart from 1 death with chlorpromazine at 256 mg/kg, none of the neuroleptics tested were toxic. Chlorpromazine, haloperidol and pimozide induced marked sedation together with decreases in traction, muscle tone and reactivity to touch with ptosis, hypothermia and myosis. Chlorpromazine appeared to the most sedative, whereas haloperidol caused clear catalepsy and abolition of the corneal reflex at 256 mg/kg. Sulpiride was without effect in the primary observation test.

Haloperidol (1 and 4 mg/kg) and sulpiride (128 mg/kg) caused

statistically significant increases in the duration of immobility with a similar but nonsignificant tendency with chlorpromazine (1 mg/kg). Pimozide was without clear effect on this parameter. None of the compounds significantly affected the power of the movements.

Pharmacologist's conclusion

Chlorpromazine: "The marked sedative phenomena observed during the primary observation test together with the tendency to increase the duration of immobility without modifying the power of movements suggest that the compound possesses neuroleptic activity."

Haloperidol: "The sedation, catalepsy, ptosis and hypothermia observed in the primary observation test together with the marked increase in duration of immobility without change in the power of the movements clearly suggest neuroleptic activity."

Pimozide: "The clear sedative effects observed during the primary observation test (together with decreases in fear, muscle tone and reactivity to touch) could suggest sedative or neuroleptic activity but the compound is without effect in the tail suspension test. Further tests would be required to confirm neuroleptic activity."

Sulpiride: "Although the compound is largely without effect in the primary observation test, the increase in the duration of immobility in the tail suspension test without change in the power of movements is a profile suggestive of neuroleptic activity. Further experiments would be required to confirm this hypothesis."

Effects of minor tranquillizers

The effects of chlordiazepoxide and diazepam are shown in Table IV.

Neither compound was toxic at 256 mg/kg. Both compounds caused marked sedation together with decreases in traction, grasping, reactivity to touch and muscle tone with motor incoordination and hypothermia at the highest doses tested. Diazepam appeared to be more potent causing ptosis and abolition of the corneal reflex at 32 and 256 mg/kg and signs of sedation still clearly present at 4 mg/kg.

Both compounds clearly increased the duration of immobility in the tail suspension test and at the same doses clearly decreased the power of the movements.

Pharmacologist's conclusion

Chlordiazepoxide: "The clear effects observed during the primary observation test, i.e. sedation, motor incoordination, loss of grasping, hypothermia without loss of the righting reflex suggest benzodiazepine-like activity.

TABLE IV

Effects of minor tranquillizers in the rapid screening procedure

Test Compound: Chlordiazepoxide

Dose mg/kg i.p.	Mortality	Primary Observation Test: Behavioural change	Primary Observation Test: Other signs
256	0/3	marked sedation decreased fear decreased traction decreased grasping decreased reactivity motor incoordination decreased muscle tone	hypothermia
64	0/3	marked sedation decreased traction decreased muscle tone	mild hypothermia
16	0/3	sedation	–

Dose mg/kg i.p.	Tail Suspension Test: Immobility (% change from control)	Tail Suspension Test: Power of movement (% change from control)
16	+137**	−61***
4	+ 63	−20

Test Compound: Diazepam

Dose mg/kg i.p.	Mortality	Primary Observation Test: Behavioural change	Primary Observation Test: Other signs
256	0/3	marked sedation decreased traction decreased grasping motor incoordination decreased muscle tone	ptosis abolition of corneal reflex marked hypothermia
32	0/3	marked sedation decreased traction decreased grasping motor incoordination decreased muscle tone	ptosis abolition of corneal reflex hypothermia
4	0/3	mild sedation decreased traction decreased grasping	mild hypothermia

Dose mg/kg i.p.	Tail Suspension Test: Immobility (% change from control)	Tail Suspension Test: Power of movement (% change from control)
4	+78*	−79***
1	+ 3	−37

Student's *t*-test: * $p < 0.05$; *** $p < 0.001$.

TABLE V

Effects of sedative/hypnotics in the rapid screening procedure

Test Compound: Barbital

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	0/3	excitation up to 15 min Afterwards marked sedation decreased traction decreased grasping motor incoordin. (15 min) loss of righting reflex sleep (30 min, 60 min) decreased muscle tone	ptosis hypothermia myosis
32	0/3	–	–

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
64	+18	−23
16	−22	− 3

Test Compound: Pentobarbital

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	3/3	–	–
32	0/3	sedation decreased fear loss of righting reflex sleep decreased muscle tone catalepsy	abolition of corneal reflex myosis
8	0/3	sedation decreased traction 1/3	–

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
8	−66**	+ 5
2	+ 2	−16

Student's *t*-test: ** $p < 0.01$.

This impression is confirmed by the results from the tail suspension test where the compound, like benzodiazepines, increased the duration of immobility and decreased the power of movements."

Diazepam: "The sedation, motor incoordination, loss of grasping and hypothermia without loss of the righting reflex suggest benzodiazepine-like activity. This impression is confirmed by the results from the tail suspension test where the compound, like benzodiazepines, increased the duration of immobility and decreased the power of movements."

Effects of sedative/hypnotics

The effects of barbital and pentobarbital are shown in Table V.

Pentobarbital but not barbital was toxic at 256 mg/kg. Both compounds caused marked sedation with motor incoordination and loss of righting reflex together with hypothermia and myosis. With barbital, unlike with pentobarbital, these effects were preceded by a short period of excitation (15 min). Pentobarbital was active at lower doses than barbital; at 8 mg/kg signs of sedation were still clearly present, whereas barbital had no more observable effects at 32 mg/kg.

Barbital was without effect on either parameter of the tail suspension test. Pentobarbital on the other hand clearly reduced the duration of immobility at 8 mg/kg without affecting the power of movements.

Pharmacologist's conclusion

Barbital: "The brief period of excitation followed by sedation and loss of the righting reflex suggest the hypnotic activity of certain barbiturates. The compound is devoid of activity in the tail suspension test."

Pentobarbital: "The marked hypoactivity, decrease in traction and muscle tone followed by loss of the righting reflex suggest sedative/hypnotic activity, whereas the decrease in immobility during the tail suspension test suggests an antidepressant component."

Effects of dopaminergic stimulants

The effects of (+)-amphetamine and apomorphine are shown in Table VI. Both compounds were toxic at 256 mg/kg. At 32 and 8 mg/kg, amphetamine caused marked behavioural excitation accompanied by fearfulness, salivation, lacrimation, hyperthermia and mydriasis. Apomorphine caused some convulsions at 32 mg/kg and clear stereotypies at 32 mg/kg and 8 mg/kg together with marked hypothermia.

In the tail suspension test, amphetamine markedly reduced the duration of immobility, particularly at the lower dose tested (2 mg/kg) and at this dose tended to increase the power of movements. Apomorphine (1 and

TABLE VI

Effects of dopaminergic stimulants in the rapid screening procedure

Test Compound: (+) Amphetamine

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	3/3	–	–
32	0/3	marked sedation salivation lacrimation increased fear decreased traction increased reactivity	hyperthermia mydriasis
8	0/3	marked excitation salivation lacrimation increased fear increased reactivity	hyperthermia mydriasis

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
8	−44	−34
2	−91***	+39

Test Compound: Apomorphine

Dose mg/kg i.p.	Mortality	Primary Observation Test	
		Behavioural change	Other signs
256	3/3	–	–
32	0/3	convulsions stereotypies	ptosis marked hypothermia
4	0/3	stereotypies	ptosis marked hypothermia

	Tail Suspension Test	
	Immobility (% change from control)	Power of movement (% change from control)
1	+35	+24
0.25	− 9	+12

Student's *t*-test: *** $p < 0.001$.

TABLE VII

Effects of dummy compounds in the rapid screening procedure

Dose mg/kg i.p.	Mortality	Primary Observation Test: Behavioural change	Primary Observation Test: Other signs
Test Compound: Glucose D (+)			
256	0/3	slight excitation (15 min)	–
		Tail Suspension Test: Immobility (% change from control)	Tail Suspension Test: Power of movement (% change from control)
128		−19	+19
32		− 8	−30
Test Compound: Sodium Bicarbonate			
256	0/3	slight excitation (15 min)	–
		Tail Suspension Test: Immobility (% change from control)	Tail Suspension Test: Power of movement (% change from control)
128		−28	−6
32		− 4	+2
Test Compound: Sodium Chloride			
256	0/3	slight excitation (15 min)	–
		Tail Suspension Test: Immobility (% change from control)	Tail Suspension Test: Power of movement (% change from control)
128		−31	−12
32		−47	+13

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0.25 mg/kg) was without effect on either parameter in the tail suspension test.

Pharmacologist's conclusion

(+)-amphetamine: "The symptoms observed during the primary observation test (excitation, salivation, lacrimation, hyperthermia, mydriasis) and the decrease in the duration of immobility in the tail suspension test clearly suggest amphetamine-like sympathomimetic activity."

Apomorphine: "Even in the absence of any activity in the tail suspension test, the clear stereotypies and profound hypothermia observed during the primary observation test clearly suggest apomorphine-like activity."

Effects of dummy compounds

The effects of glucose D(+), sodium bicarbonate and sodium chloride are shown in Table VII.

None of the compounds were toxic or produced any behavioural signs at 256 mg/kg apart from a transient excitation 15 min after the injection.

They showed no activity in the tail suspension test apart from sodium chloride which tended to decrease the duration of immobility at both doses tested, an effect which was close to statistical significance at 32 mg/kg.

Pharmacologist's conclusion

Glucose D(+): "The compound is nontoxic and devoid of psychotropic activity in the behavioural procedures investigated."

Sodium bicarbonate: "The compound is nontoxic and devoid of psychotropic activity in the behavioural procedures investigated."

Sodium chloride: "The compound is nontoxic and has no behavioural effects in the primary observation test. The tendency to decrease the duration of immobility in the tail suspension test could suggest antidepressant activity but would have to be confirmed."

Discussion

The aim of the present experiments was to see whether the pharmacological activity of different psychotropic compounds could be correctly identified using a screening procedure combining elements of an Irwin-style primary observation test (Irwin, 1968) with the automated tail suspension test–ITEMATIC-TST (Stéru *et al.*, 1985, 1986; Porsolt *et al.*, 1986). To increase the credibility of the findings, all experiments were conducted blind in simulated real life testing conditions.

The results obtained indicate that the procedures adopted were capable of selecting an appropriate dose-range and of identifying the phar-

macological activity of the majority of the compounds investigated, without falsely ascribing pharmacological activity to the dummy compounds included in the study. Desipramine, imipramine and viloxazine were clearly identified as antidepressants (sedation in the primary observation test, decreased immobility in the tail suspension test) with a similar but statistically nonsignificant tendency being observed with the 2 monoamine oxidase inhibitors (clorgyline, nialamide). Even if the effects observed in the tail suspension test were slight, the effects observed in the primary observation test appeared sufficient to warrant further investigation of these 2 compounds which were clearly not devoid of psychotropic activity. Similarly to the tricyclic antidepressants, chlorpromazine and haloperidol were clearly identified as neuroleptics on the basis of their effects in the primary observation test (marked sedation) and their profile in the tail suspension test (increased immobility, unchanged power of movements). A similar profile was observed with sulpiride in the absence of effects in the primary observation test, whereas pimozide possessed no clear profile in the tail suspension test, but induced marked sedation in the primary observation test, suggestive of eventual neuroleptic activity. The pharmacological activity of the 2 benzodiazepines could also be correctly identified both on the basis of the primary observation test (sedation, muscular relaxation, motor incoordination) and the tail suspension test (increased immobility and decreased power of the movements). Similarly the sympathomimetic and stimulant activity of amphetamine was identifiable both from the excitation observed during the primary observation test and the decrease in immobility in the tail suspension test. Amphetamine could be clearly distinguished from apomorphine which induced stereotypies and hypothermia in the primary observation test, but was without effect in the tail suspension test. The hypnotic activity of the 2 barbiturates (barbital and pentobarbital) was clearly apparent from the primary observation test and the 2 compounds could be further distinguished from their activity profiles in the 2 procedures; barbital induced a short-lasting but marked behavioural excitation and was inactive in the tail suspension test, whereas pentobarbital induced only sedation and decreased the immobility in the tail suspension test. Finally, none of the dummy compounds tested showed any activity in either test procedure, apart from NaCl which showed a nonsignificant tendency to reduce the duration of immobility in the tail suspension test.

Taken together, these results present an interesting interplay between the effects observed in the primary observation test and in the tail suspension test. The pharmacological activity of some compounds (e.g. desipramine, imipramine, viloxazine, sulpiride) could be identified mainly in terms of their effects in the tail suspension test, whereas the activity of other compounds (e.g. amphetamine, apomorphine, the barbiturates) was identifiable mainly in terms of their effects in the primary observation test. For the

classical neuroleptics and the other benzodiazepines, on the other hand, clear profiles were obtained in both tests. The main point, however, is that the combined use of both procedures permitted unambiguous identification of the pharmacological activity (or its absence) in 13 of the 18 compounds tested with tentative but correct identification of the pharmacological activity of a further 2 (pimozide, sulpiride). Of the 3 remaining compounds, clorgyline and nialamide, were clearly not devoid of psychotropic activity, whereas NaCl had no observable effects apart from a nonsignificant tendency to reduce immobility in the tail suspension test.

The present findings largely confirm and extend those reported in our previous papers (Stéru *et al.*, 1985b, 1986; Porsolt, 1986) concerning the activity profiles in the tail suspension test of classical antidepressants, psychostimulants, neuroleptics and benzodiazepine minor tranquillizers. One potentially discordant finding is the failure of the 2 monoamine oxidase inhibitors clorgyline and nialamide to significantly decrease the duration of immobility in the present experiments. This may have been due to the fact that in the present experiments a 30 min injection time was used instead of the 60 min injection time employed in the previous study (Stéru *et al.*, 1986). Our general experience, however, suggests that monoamine oxidase inhibitors have less robust effects in the tail suspension test than tricyclics and other antidepressants. A further finding of interest in the present experiments was the decrease in immobility observed with pentobarbital. Although this compound is not known for antidepressant activity, it can be noted that a similar effect has been observed at similar doses in the traditional "behavioural despair" test (Schechter and Chance, 1979).

In conclusion, the present experiments have indicated that the combined use of the tail suspension test with a primary observation procedure can correctly identify the psychotropic activity of a wide variety of different compounds. Further experience will show whether other profiles can be established for a greater range of psychotropic activities, but it seems unlikely that the procedures described here would miss these activities altogether. It must be emphasized that the present procedures in no way substitute for the complete battery of psychopharmacological tests necessary for characterizing and confirming the activity of a novel compound. Nonetheless, we suggest that the rapid behavioural procedures we have described would be eminently suitable for the primary screening of potential psychotropic agents.

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The tail suspension test: A new method for screening antidepressants in mice

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Abstract. A novel test procedure for antidepressants was designed in which a mouse is suspended by the tail from a lever, the movements of the animal being recorded. The total duration of the test (6 min) can be divided into periods of agitation and immobility. Several psychotropic drugs were studied: amphetamine, amitriptyline, atropine, desipramine, mianserin, nomifensine and viloxazine. Antidepressant drugs decrease the duration of immobility, as do psychostimulants and atropine. If coupled with measurement of locomotor activity in different conditions, the test can separate the locomotor stimulant doses from antidepressant doses. Diazepam increases the duration of immobility.

The main advantages of this procedure are (1) the use of a simple, objective test situation, (2) the concordance of the results with the validated "behavioral despair" test from Porsolt and, (3) the sensitivity to a wide range of drug doses.

Key words: Immobility test – Antidepressants – Screening method – Mice

Porsolt (1981) proposed a model for screening antidepressants in mice, called "behavioral despair". In this test, a mouse placed in water swims, apparently trying to escape; it then alternates swimming and immobility periods. Antidepressants (and some other drugs) reduce the immobility periods.

We report here the results of a new procedure, inspired by Porsolt's test situation, and based on a concept (the "searching-waiting strategy") which is described elsewhere (Stéru et al. 1982; Thierry et al. 1984).

From a theoretical point of view, this test supports the following hypothesis: a normal animal submitted to an insoluble, aversive situation alternates between two kinds of behaviors, agitation, and immobility. These can be named *searching-behavior* characterized by intense motor activity and expense of energy, and *waiting-behavior* with immobility and energy saving. The choice sequences between these kinds of behaviors can be named as the *searching-waiting strategy*. The following data support the assumption that antidepressant drugs modify the balance between these forms of behavior in the favour of searching.

Offprint requests to: P. Simon

Materials and methods

The subjects were naive male NMRI mice (from Centre d'Elevage Roger Janvier, France), weight 22–24 g. The animals were housed in plastic cages in groups of ten per cage, at room temperature about 21 ± 1° C, and with free access to water and food. They were kept on an artificial 12 h/12 h day/night cycle.

The method is based on the observation that a mouse suspended by the tail shows alternate periods of agitation and immobility. For these experiments, the recording device was as follows: metallic gallows were connected to a nylon catheter (d = 1.5 mm, length = 350 mm) with a hook attached to its extremity. The distance between the floor of the device and the hook was 350 mm. The mouse was hung on the hook by an adhesive tape placed 20 mm from the extremity of its tail.

The mouse was 150 mm away from the nearest object and was both acoustically and visually isolated. The articulated stylus of the gallows was connected to a Marey capsula that transmitted any pressure difference to another capsula by a pneumatic connection. The receiver capsula was connected to a drawing stylus, marking on a cylinder covered with black smoke. The cylinder rotated at 2 cm/min regulated by an electric motor. This device provided an analogue record of the movements of the mouse. The device was set in order to ignore respiratory movements, and recorded only body movements.

On the recording (see sample on Fig. 1) it is easy to measure the length of immobility (flat recording), and to convert it to the duration of immobility. These measurements were always made under blind conditions.

The recording duration was 6 min.

Experimental procedure. Each group was composed of 10 or 20 mice. Control mice were given distilled water (D. W.) IP: 0.25 ml/20 g body weight, 30 min before test, except for controls for the amitriptyline group, which received D. W. 60 min before testing. All mice were isolated in plastic boxes (20 × 10 × 10 cm), between the injection and the test.

Treated mice where given the following drugs IP: *d*-amphetamine sulfate (Cooperative Pharmaceutique Française); amitriptyline hydrochloride (Roche); atropine sulfate (Sigma); desipramine hydrochloride (Ciba-Geigy); diazepam (Roche); imipramine hydrochloride (Ciba-Geigy); imipramine methiodide (Ciba-Geigy); mianserin hydrochloride (Organon); nomifensine maleate (Hoechst); viloxazine hydrochloride (1. C. I.-Pharma). The vehicle for

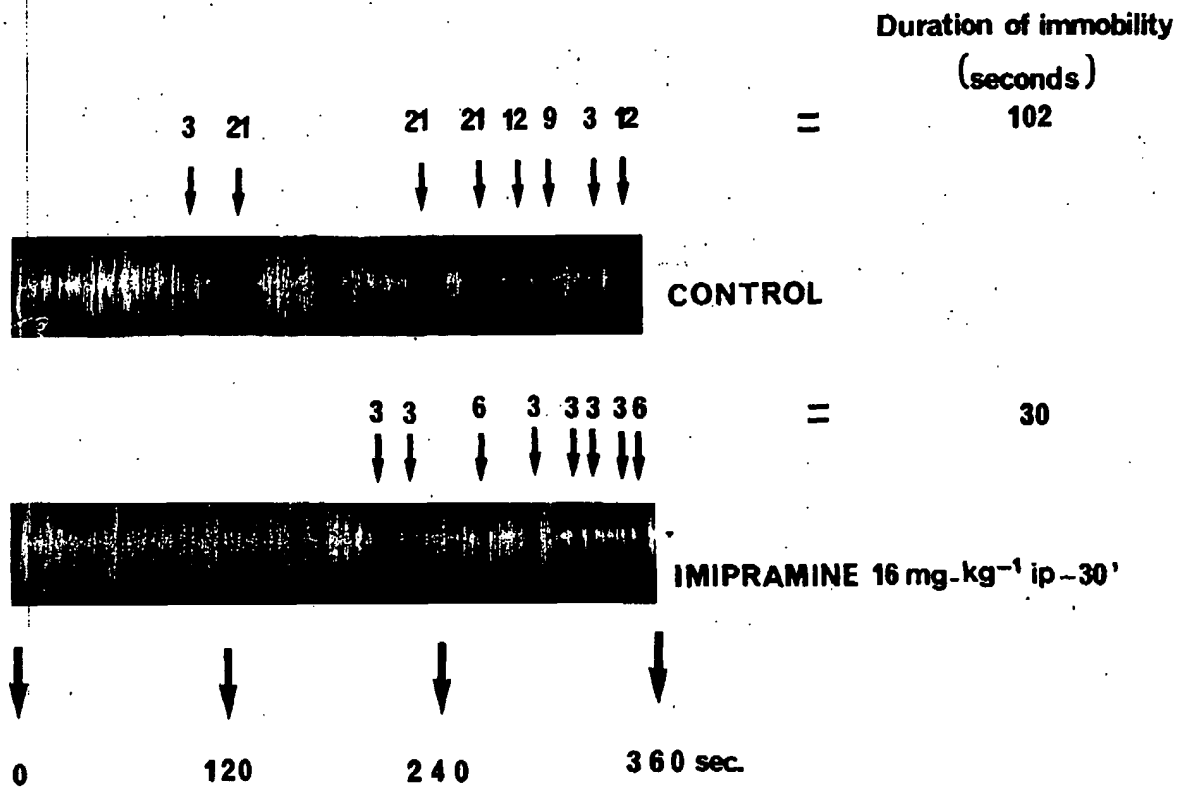

Fig. 1. Recording of a control and a 16 mg · kg^{-1} imipramine-treated mouse. The *arrows* indicate the immobility periods (s), the total immobility duration being summed on the right side

the injected drugs was distilled water or an acacia gum suspension for the non-soluble drugs.

Statistical procedures. Comparisons of the mean duration of immobility (in s) were performed using analysis of variance (ANOVA); those between the various treatment groups were performed using the Dunnett test.

Results

Behavior of control animals. Observation of the controls suggested that the mice suspended to the recording device by the tail made apparent escape efforts which could be classified into three types: (1) running movements, forward or backwards; (2) body torsion with attempts to catch the suspending bond; (3) body jerks.

After several attempts, the mice stopped moving and hung motionless. The agitation testing periods which continued to be performed were separated by longer or more frequent periods of immobility.

The pooled results obtained with 380 control mice, receiving D. W. 30 min before testing are shown in Fig. 2, which provides a frequency histogram of the distribution of the duration of immobility. During the different tests performed, 38 groups of 10 controls were studied. The mean duration of immobility was three times less than or equal to 60 s; seven times between 60 and 70 s, 16 times between 70 and 80 s, eight times between 80 and 90 s, three times between 100 and 110 s and once over 110 s.

Effects of drugs. Table 1 shows that imipramine, desipramine, and amitriptyline each produced a significant dose related reduction of immobility [$F(7,14) = 10.38$, $P < 0.001$ for imipramine; $F(8,12) = 6.69$, $P < 0.001$ for desipramine; $F(7,12) = 8.43$, $P < 0.001$ for amitriptyline].

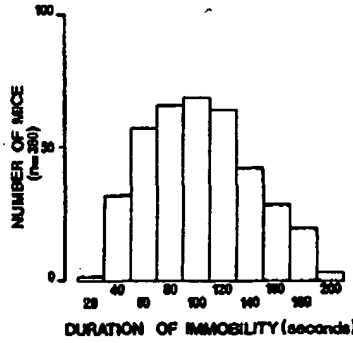

Fig. 2. Duration of immobility: frequency distribution of 38 groups of control mice

Imipramine methiodide, a quaternary ammonium that crosses the blood-brain barrier poorly, had no effect upon the duration of immobility, even at the highest dose; at this dose (32 mg/kg) three out of 10 mice died.

Table 2 shows that mianserin and viloxazine decreased the duration of immobility [$F(5,64) = 3.94$, $P < 0.001$ for mianserin; $F(4,45) = 10.11$, $P < 0.001$ for viloxazine]. However, for mianserin, there was a non linear dose-effect relationship. Nomifensine reduced immobility from a dose of 0.06 mg/kg [$F(9,23) = 20.12$, $P < 0.001$].

Table 3 shows that higher doses of atropine reduced immobility, as did amphetamine, at the higher doses. At lower doses, amphetamine increased immobility time, significantly at 0.25 mg/kg [$F(3,36) = 6.12$, $P < 0.001$ for atropine; $F(7,10) = 9.70$, $P < 0.001$ for amphetamine].

Table 1. Effect of tricyclic antidepressants and derivatives upon the duration of immobility. (Number of seconds during the 6 min duration of the test).

	0	0.125	0.5	1	2	4	8	16	32
Imipramine	102 ± 7.8		83.4 ± 14.4	70.2 ± 16.5	58.5 ± 7.9[b]	52.6 ± 10.9[b]	48.1 ± 9.8[b]	27.5 ± 6.5	19.6 ± 6[b]
Desipramine	94.0 ± 6.0	66.9 ± 16 NS	64.8 ± 15 NS	38.1 ± 10[b]	41.2 ± 8[b]	41.4 ± 12[b]	40.5 ± 11[b]	36.5 ± 10[b]	35.4 ± 7[b]
Amitriptyline	96.8 ± 7.0		59.0 ± 16 NS	44.4 ± 13[b]	57.0 ± 9.2[a]	59.7 ± 13 NS	44.7 ± 11[b]	26.5 ± 8[b]	32.7 ± 7[b]
Imipramine methiodide	80.7 ± 11			86.7 ± 14.0	64.8 ± 12.1	85.2 ± 10	68.7 ± 16	71.7 ± 12	104.7 ± 20 3 deaths

Drugs were injected IP 30 min before test expected for amitriptyline injected IP 60 min before test. NS Not significant, [a] $P < 0.05$, [b] $P < 0.01$ (Dunnett's test)

Table 2. Effect of atypical antidepressants upon the duration of immobility. (Number of seconds during the 6 min duration of the test)

	0	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64
Mianserin	86.2 ± 8.4								69.0 ± 12 NS	44.8 ± 6.8[b]	44.4 ± 6.7[a]	49.2 ± 11[a]	59.1 ± 7.7 NS
Viloxazine	96.6 ± 10								61.2 ± 16 NS	34.5 ± 9[b]	32.4 ± 8[b]	12.6 ± 4[b]	
Nomifensine	90.5 ± 6	102 ± 18	59.4 ± 9[a]	36.6 ± 6[b]	37.0 ± 8[b]	40.6 ± 6[b]	30.5 ± 6[b]	24.5 ± 4[b]	18.0 ± 5[b]	4.5 ± 2[b]			

Drugs were injected IP 30 min before test. NS Not significant, [a] $P < 0.05$, [b] $P < 0.01$ (Dunnett's test)

Table 3. Effect of different drugs upon the duration of immobility. (Number of seconds during the 6 min duration of the test)

	0	0.06	0.125	0.25	0.5	1	2	4	8	16
Dexamphetamine	97 ± 7	105.6 ± 17	117 ± 11	126 ± 8 NS	69.3 ± 9	72.3 ± 13	29.1 ± 12[b]	27.0 ± 7[b]		
Atropine	99.6 ± 11						84.6 ± 18	38.1 ± 6.6[b]		47.1 ± 8.4[b]
Diazepam	92.4 ± 11				105 ± 16		160 ± 17[b]		220 ± 19[b]	

Drugs were injected IP 30 min before test. NS Not significant, [a] $P < 0.05$, [b] $P < 0.01$ (Dunnett's test)

Table 4. Effect of desipramine (16 mg/kg) in chronic administration (twice a day, for 7 days). (Number of seconds during the 6 min duration of the test)

Chronic treatment (twice a day, 7 days)	Acute treatment (inj. 30 min before test)	Duration of immobility (s ± SEM)
D. W.	D. W.	70.2 ± 15
Desipramine	Desipramine	13.3 ± 2.2[b]
Desipramine	D. W.	101 ± 16
D. W.	Desipramine	28.0 ± 7.3[a]
No treatment	No treatment	64.0 ± 15

D. W. = distilled water, [a] $P < 0.05$, (Dunnett's test), [b] $P < 0.01$

Diazepam increased the duration of immobility at 0.5, 2, and 8 mg/kg [$F(3,46) = 14.50$, $P < 0.001$].

Effect of 7 days' administration of desipramine. When desipramine was injected twice a day for 7 days, there was no significant decrease in immobility when the last injection was given 12 h before the test. When there was one more injection 30 min before the test, the decrease in immobility was greater (but not significantly) than when there was only an acute treatment with the same dose of desipramine. It appears that the chronic treatment with desipramine does not diminish the acute effect of the drug on this test (and perhaps increases it).

Discussion

Action of antidepressants. All of the above experiments showed that every antidepressant studied decreased immobility, in terms of duration. However, this test does not measure merely locomotor stimulation, as can be seen from two arguments:

1) The sedative antidepressants (such as amitriptyline and mianserin) decrease immobility at doses previously shown to be sedative for locomotor activity (Porsolt et al. 1978).

2) The locomotor stimulatory antidepressants, such as nomifensine, decrease the duration of immobility at doses

that are clearly smaller than the locomotor stimulant doses (Hoffman 1973). It can be concluded that the tail-suspension test can dissociate the locomotor stimulant from the antidepressant effects of antidepressants, when the latter occurs at lower doses. Clear dose-effect relationships were found with amitriptyline, desipramine, imipramine, nomifensine, viloxazine, and, in the opposite direction, with diazepam.

One could emphasize that in a really satisfactory model of depression, acute administration of drugs should not be active, as far as repeated treatment administrations are necessary in human patients. We cannot explain why acute drug effects are observed.

The lack of effect of imipramine methiodide suggests that the action of the antidepressants on this test is mediated by a central mechanism.

No linear dose-effect relationship was found with two drugs, mianserin and amphetamine. For mianserin, the locomotor sedative effect may mask the reduction of immobility. For amphetamine, the dose-effect curve seems to be biphasic. It is known (Simon 1970) that amphetamine at low doses decreases locomotor activity and may have an anxiogenic effect, which accounts for the enhanced, "freezing"-like immobilisation of mice in the tail-suspension test. Another possible explanation is that low doses of amphetamine decrease catecholamine availability in brain (Huang and Maas 1981), and the tail suspension test may interfere with catecholamines, in the sense that raised levels of catecholamines lead, to a decrease in tail suspension induced immobility. Further experiments will be designed to verify this.

The reduction in immobility time does not seem to be specific to clinically active antidepressants: atropine and amphetamine also reduce immobility in the test. However, both drugs have been described as having a clear stimulant effect in animals, and a potential antidepressant effect in man. Indeed for both drugs, Porsolt (1981) reported similar results using his test.

Chronic (twice a day for 7 days) administration was not very effective in modifying behavior on this test when the last injection was given 12 h before the test. One possible explanation of the difference between the effects of chronic administration in man and rat can be accounted for by pharmacokinetics.

Half-life is about 70 min, in brain and blood in mice, which is very different from that in humans (12–77 h) (Diquet et al. submitted). However, it should be noted that if acute administration is preceded by 2 weeks' administration of the same drug, the acute effect is not diminished and even increased – although not significantly – unlike in other tests. These preliminary results are in good agreement with the results reported by Porsolt with his test (1981).

Two main differences between the Tail Suspension Test and Porsolt's test can be listed. First, the immersion, which is necessary to produce the "behavioral despair", induces a deep hypothermia in mice (personal unpublished observations for mice; for rats, Porsolt et al. 1979); this is avoided in the Tail Suspension Test. A second point is the recording of an objective measure in the Tail Suspension Test, which might be more precise than the human observation on which is based the appreciation of immobility in the "behavioral despair" test. The third difference between the two tests is that the Tail Suspension Test is more sensitive to lower doses of drug and provides, as might be suggested in some cases by these preliminary results, a clearer dose-effect relationship.

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THE AUTOMATED TAIL SUSPENSION TEST: A COMPUTERIZED DEVICE WHICH DIFFERENTIATES PSYCHOTROPIC DRUGS

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(Final form, June 1987)

Abstract

Stéru Lucien, Raymond Chermat, Bernard Thierry , Juan Antonio Mico, Antoine Lenègre, Marius Stéru, Pierre Simon and Roger D. Porsolt: The automated Tail Suspension Test : A computerized device which differentiates psychotropic drugs. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 1987, 11 : 659-671.

1. Mice when suspended by the tail will alternate between active attempts to escape and immobility. Immobility like that measured in the behavioral despair test is reduced by a wide variety of antidepressant agents.
2. The present paper describes a computerized version of this test (ITEMATIC-TST) which in addition to recording immobility measures the power of the movements.
3. Various tricyclic (amitriptyline, desipramine, imipramine), MAOI (clorgyline, moclobemide, nialamide, pargyline, toloxatone) and atypical antidepressants (bupropion, citalopram, indalpine, mianserin, nomifensine, viloxazine) were tested and compared with psychostimulants (d-amphetamine, caffeine), neuroleptics (chlorpromazine, haloperidol, sulpiride), anxiolytics (clobazam, diazepam) and agents acting on the cholinergic system (atropine, oxotremorine).
4. All antidrepressants decreased the duration of immobility and most increased the power of movements.
5. The psychostimulants also decreased immobility but only amphetamine increased the power of movements.
6. Neuroleptics increased immobility without affecting the power of movements, whereas anxiolytics increased immobility but decreased the power of movements.
7. Atropine had a profile similar to antidepressants whereas oxotremorine tended to have opposite effects.
8. The results suggest that the automated test system with its two parameters is not only sensitive to antidepressants but could also be useful for generating activity profiles for different kinds of psychotropic agent.

Keywords : antidepressants, immobility test, mice, psychotropic drugs, screening profile.

Abbreviations : monoamine oxidase (MAO), monoamine oxidase inhibitors (MAOI)

Introduction

Animals when placed in an aversive situation from which there is no escape will alternate between two behaviors : vigorous activity and immobility ("despair"). The immobile behavior induced in rodents by forcing them to swim in a restricted space has been proposed by one of us as a screening model for testing antidepressants and has been found sensitive to a wide variety of antidepressant agents including tricyclics, MAOI and atypical drugs such as mianserin, iprindole and viloxazine (Porsolt 1981). More recently, a similar phenomenon of immobility has been described in mice which are suspended by the tail (Stéru et al 1985a, Thierry et al 1984). Like behavioral despair, tail suspension-induced immobility is particularly sensitive to antidepressant treatments.

The original tail suspension method made use of a smoked drum to record periods of activity and immobility which were then scored manually. This procedure was very time consuming and laborious. The present paper describes the results obtained with a newly developed computerized device (ITEMATIC-TST) (Stéru et al 1985b ; Mico et al 1986) which in addition to recording the duration of immobility measures the power of the individual movements. This automatic apparatus not only provides an objective measure of behavioral changes occurring in this situation but also vastly increases the number of animals which can be tested by a single experimenter. Furthermore the measurement of two parameters, immobility and power of the movements, permits identification of different classes of psychotropic compound by means of their activity profiles in this primary screening test.

Material and Methods

Subjects

Naive male NMRI mice (from the Centre d'Elevage Roger Janvier, France) weighing 22 - 24 g were used. The animals were housed in plastic cages in groups of 10 per cage at a temperature of 21 ± 1° C with free access to water and food. An artificial non-reversed 12/12 h day/night cycle was imposed.

Drugs

The following drugs were investigated : amitriptyline hydrochloride (Roche), d-amphetamine sulphate (Coopérative Pharmaceutique Française), atropine sulphate (Sigma), bupropion hydrochloride (Wellcome), caffeine (Aldrich), chlorpromazine hydrochloride (Rhône Poulenc), citalopram hydrochloride (Lundbeck), clobazam (Diamant), clorgyline hydrochloride (May and Baker), desipramine hydrochloride (Ciba-Geigy), diazepam (Roche), haloperidol (Janssen), imipramine hydrochloride (Ciba-Geigy), imipramine methiodide (Ciba-Geigy), indalpine hydrochloride (Pharmuka), mianserin hydrochloride (Organon), moclobemide hydrochloride (Roche), nomifensine maleate (Hoechst), oxotremorine fumarate (Sigma), pargyline hydrochloride (Sigma), sulpiride (Delagrange), toloxatone (Delalande) and viloxazine hydrochloride (ICI).

Apparatus

The experiments were performed using the automated Tail Suspension apparatus ITEMATIC-TST. This new computerized device, developed and marketed by I.T.E.M-LABO (Paris), enables 6 mice to be tested simultaneously (Figure 1). Each mouse is suspended by the tail using adhesive tape to a hook connected to a strain gauge. The strain gauge picks up all movements of the mouse and transmits them to a central unit which digitalizes the signals. The signals are displayed visually using LEDs which permit on-line verification of the good functioning of each unit. Included in the central unit is a 9 level filtering device which can be set to the desired sensitivity to provide maximum discrimination of gross body movements from other micro-movements of the animal or its internal organs. The central processing unit calculates two parameters : the duration of immobility and the power of the movements. The duration of immobility is calculated from the cumulated time during which the animals movements do not exceed the threshold determined by the 9 level filtering device. The power of the movements is calculated from the total energy expended by the animal during the test as measured by the cumulated amplitudes of individual movements (arbitrary units) divided by the total time the animal is active. The central unitis connected to a microcomputer (Epson HX20) which provides on-line data collection and analysis including generation of the experimental schedule (randomisation), grouping of results, statistical analysis and graphical and numerical presentation.

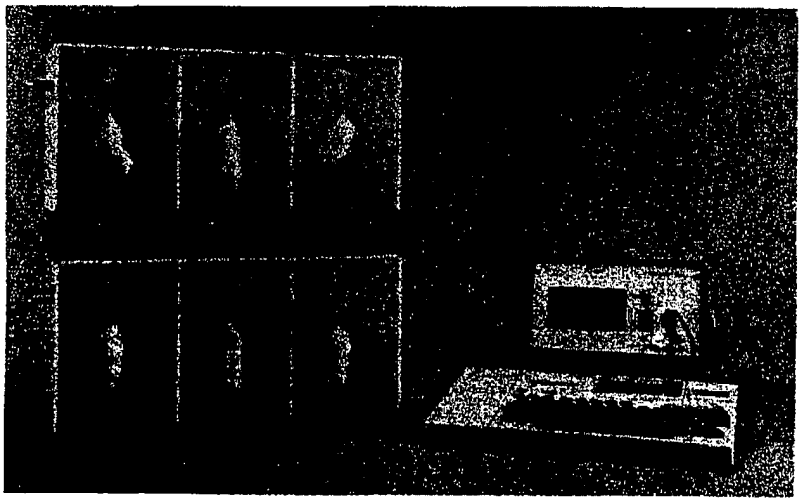

Fig. 1 - The ITEMATIC-TST with two suspension units superimposed (left), a central processing unit with LED displays (upper right) and an Epson HX20 microcomputer with extended memory (lower right).

Procedure

Animals were injected according to the experimental plan generated by the ITEMATIC-TST and were placed into individual plexiglass boxes (20 x 50 x 10 cm) between injection and the test. All drugs were injected i.p. 30 minutes before testing with the exception of amitriptyline, clorgyline, moclobemide, nialamide, pargyline and toloxatone where the injection time was 60 minutes. Moclobemide and toloxatone were administered p.o.. The test lasted 6 minutes and the measurements were performed for the total duration of the test. Soluble drugs were dissolved in distilled water and non-soluble drugs were dispersed in a suspension of acacia gum (5 %). Control animals received the vehicle. A concentration of 0.25 ml/20 g body weight was used throughout. Doses are expressed in terms of the salt. Ten animals were studied per group with the exception of mianserin and toloxatone where groups contained 20 animals.

Statistical Analyses

Differences between treated groups and their controls were analysed for statistical significance using Dunnett's t-test.

Results

Results in control animals

Control results were obtained in a total of 260 animals divided into 22 groups of 10 animals and 2 groups (the controls for mianserin and toloxatone) of 20 animals. The mean duration of immobility for all control groups combined was 89.1 seconds with individual control group means ranging from 56 to 116 seconds. About 75 % of the control means for immobility were obtained in the range 80 - 107 seconds. The mean power of the movements for the same control groups was 19.6 (arbitrary units) with individual control group means ranging from 13 to 32. Over 75 % of the control means for power of the movements were obtained in the range 13 to 24.

Tricyclic antidepressants

Amitriptyline, desipramine and imipramine all induced clear and dose-dependent decreases in the duration of immobility. They also tended to increase the power of the movements with statistically significant effects at least at one dose. A clear peak increase in the power of the movements was observed with amitriptyline at 8 mg/kg, followed at higher doses by a decline towards control levels. This decline is probably associated with the marked sedative/muscle relaxant effects observed at 16 and 32 mg/kg amitriptyline. In contrast to the tricyclic antidepressants imipramine-methiodode, a quaternary ammonium salt that crosses the blood brain barrier poorly, had no effect up to 16 mg/kg but tended to increase immobility and decrease power at 32 mg/kg. (Fig 2)

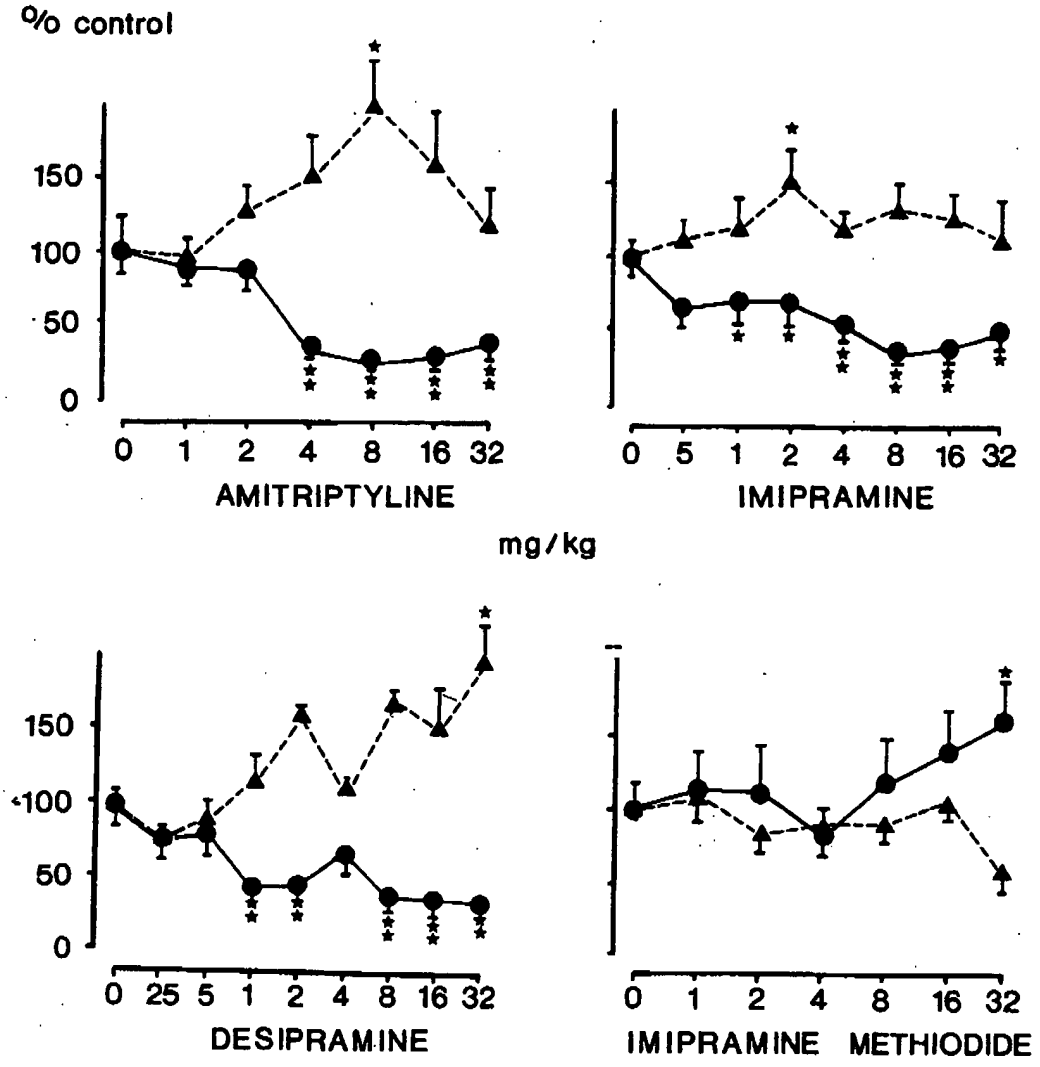

Fig. 2 - The effects of 3 tricyclic antidepressants (amitriptyline, desipramine and amipramine) and the quaternary ammonium salt imipramine methiodide on the duration of immobility (●——●) and the power of the movements (▲——▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test except for amitriptyline which was administered i.p. 60 minutes before the test. * $p < .05$; ** $p < .01$

Monoamine Oxidase Inhibitors

Clorgyline, a specific inhibitor of type A MAO, caused clear and dose-dependent decreases in immobility without significant effects on the power of the movements up to the highest dose tested (16 mg/kg). Similar but non dose-dependent effects on immobility were observed with the two reversible type A MAOI moclobemide and toloxatone, again without any significant effects on the power of the movements. Pargyline, a specific inhibitor of type B MAO, had no consistent effect on either parameter up to 128 mg/kg but significantly decreased immobility and increased power at 256 mg/kg. Nialamide, a mixed MAOI, tended to decrease the duration of immobility at all doses tested ; this effect was not dose-dependent, however, and a significant reduction was only observed at the lowest dose (4 mg/kg). The power of the movements was not affected. (Fig 3)

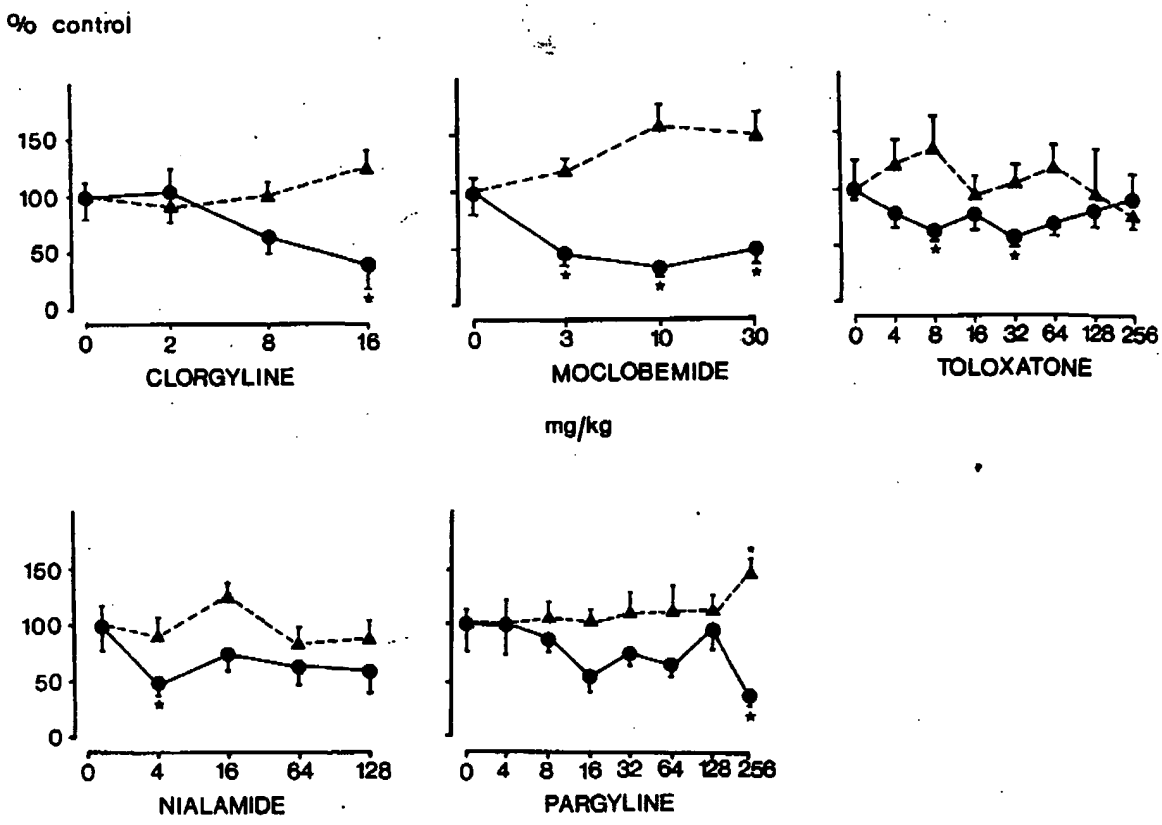

Fig. 3 - Effects of five MAO inhibitors (clorgyline, moclobemide nialamide, pargyline, toloxatone) on the duration of immobility (●—●) and the power of the movements (▲- -▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 60 minutes before the test except moclobemide and toloxatone which were administered p.o. 60 minutes before testing. * $p < .05$; ** $p < .01$

Atypical antidepressants

All the atypical antidepressants tested (bupropion, citalopram, indalpine, mianserin, nomifensine, viloxazine) caused significant decreases in the duration of immobility. These effects were dose-dependent except for mianserin where a biphasic effect was observed, first a decrease followed by a return towards control levels. This effect cannot be considered very robust as 20 animals were required to obtain statistically significant results. Bupropion, mianserin and viloxazine significantly increased the power of the movements with a similar but non-significant tendency with nomifensine. In contrast, citalopram and indalpine, two specific inhibitors of the uptake of serotonin, had no effects on the power of the movements except indalpine at the highest dose tested (64 mg/kg). (Fig 4)

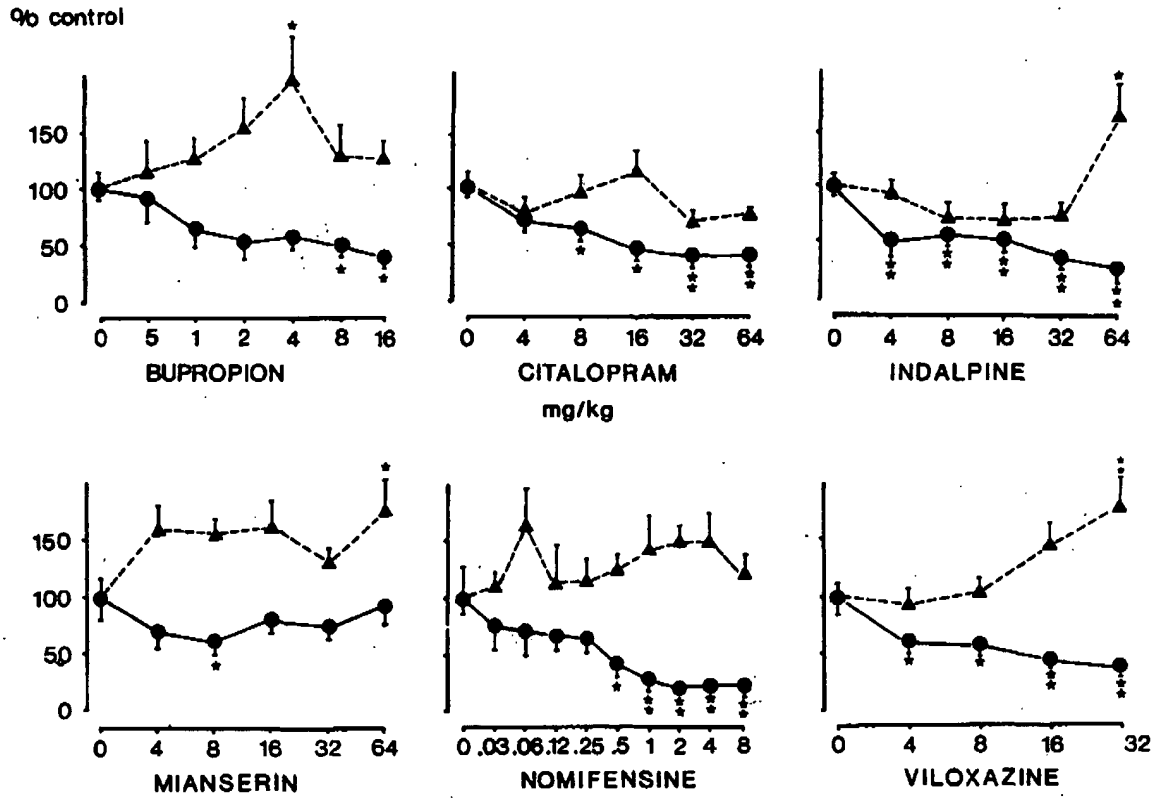

Fig. 4 - Effects of six atypical antidepressants (bupropion, citalopram, indalpine, mianserin, nomifensine, viloxazine) on the duration of immobility (●——●) and the power of the movements (▲– – –▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

Psychostimulants

Both amphetamine and caffeine caused significant decreases in the duration of immobility. These effects were largely dose-dependent except at the highest doses where a return to control values was observed. With amphetamine at 8 mg/kg, clear stereotypies were present. Caffeine had no effect on the power of the movements, whereas amphetamine increased the power of the movements up to doses inducing stereotypies. (Fig 5)

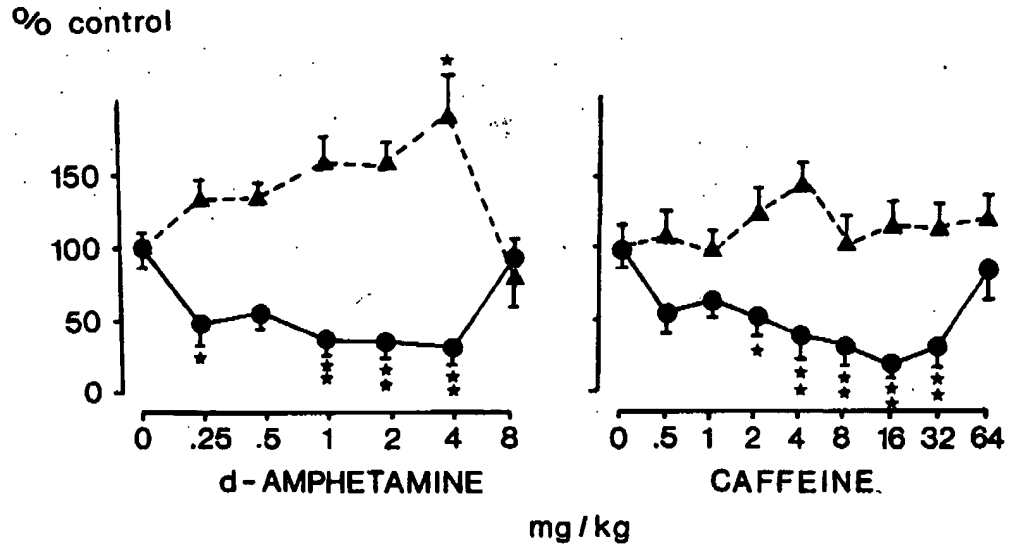

Fig. 5 - Effects of two psychostimulants (d-amphetamine, caffeine) on the duration of immobility (●——●) and the power of the movements (▲- - -▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

Neuroleptics and Minor Tranquilizers

In contrast to the antidepressants and psychostimulants, all three neuroleptics tested, chlorpromazine, haloperidol and sulpiride, dose-dependently increased the duration of immobility but had no significant effects on the power of the movements in the dose ranges tested. Similar increases in the duration of immobility were observed with the two minor tranquilizers tested (diazepam, clobazam) but these compounds, in contrast to the neuroleptics, caused significant dose-dependent decreases in the power of the movements. The decreases in the power of the movements at the higher doses were associated with clear signs of myorelaxation. (Fig 6)

Drugs Acting on the Cholinergic System

The cholinergic receptor blocker atropine significantly decreased the duration of immobility with a similar effect being observed at all doses tested (2 - 16 mg/kg). Atropine tended to increase the power of the movements but a significant effect was only observed at the lowest dose (2 mg/kg) with a return towards control values at higher doses. Oxotremorine, a cholinergic receptor agonist, had little effect on the parameters measured apart from a small but significant decrease in the power of the movements at 1 mg/kg accompanied at this one dose by a non-significant increase in the duration of immobility. (Fig 7)

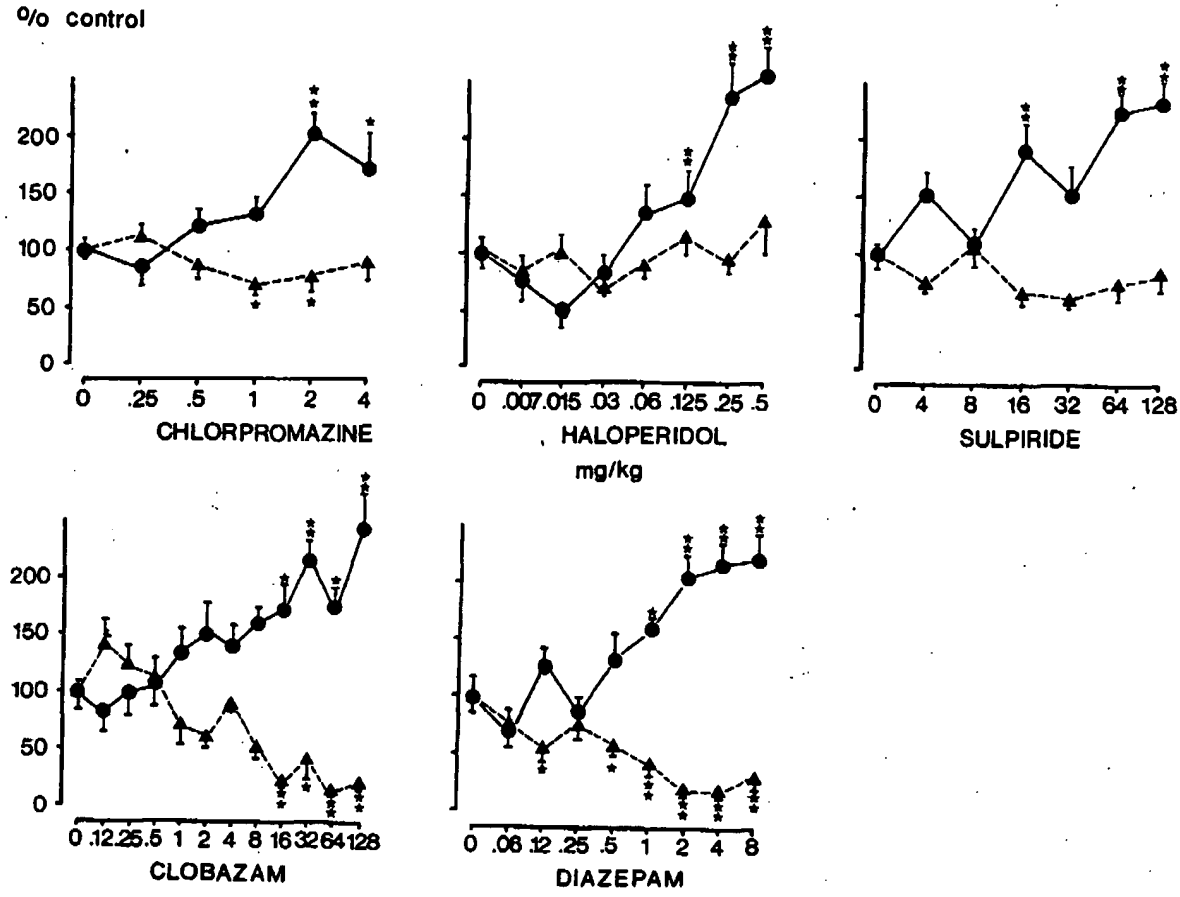

Fig. 6 - Effects of three neuroleptics (chlorpromazine, haloperidol, sulpiride) and two minor tranquilizers (clobazam, diazepam) on the duration of immobility (●——●) and the power of the movements (▲- - -▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

Discussion

Effectiveness of antidepressants

The results of these experiments have shown that a wide variety of antidepressants with differing mechanisms of action decrease the duration of immobility in the Tail Suspension Test as measured automatically using the ITEMATIC-TST. Three typical tricyclic compounds (amitriptyline, desipramine, imipramine) were clearly active whereas the quaternary ammonium salt of imipramine,

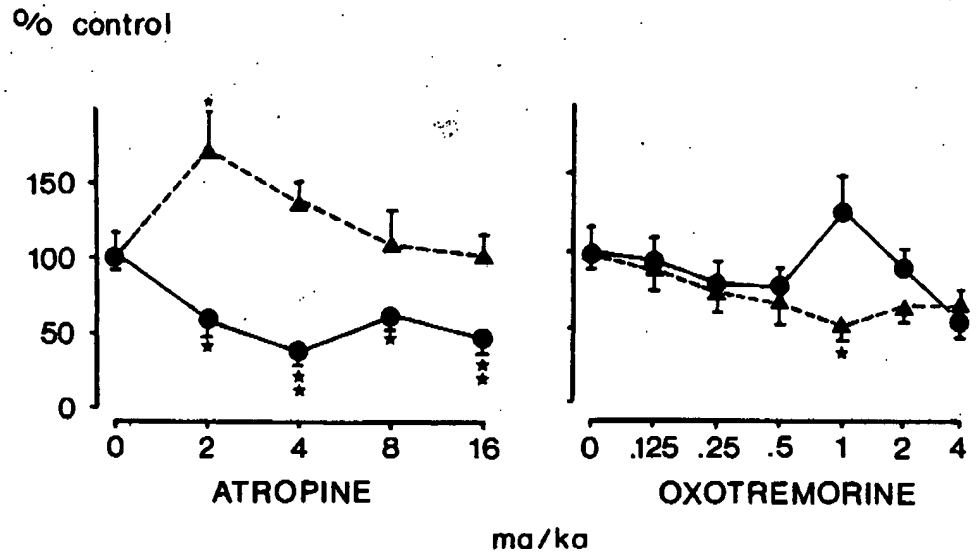

Fig. 7 - Effects of a cholinergic antagonist (atropine) and agonist (oxotremorine) on the duration of immobility (●—●) and the power of the movements (▲—▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

imipramine methiodide, which passes the blood brain barrier only poorly, had little effect in the same dose range. This suggests that the effects observed with amitriptyline, desipramine and imipramine were central in origin. Of interest also were the results obtained with the MAOI. Clorgyline, moclobemide and toloxatone, specific inhibitors of type A MAO (Neff and Fuentes 1976), were clearly active whereas pargyline, a specific inhibitor of type B MAO at low doses (Neff and Fuentes 1976), was only active at a very high and probably non-specific dose with intermediate results being obtained with the mixed type MAOI nialamide. Further compounds would need to be tested before concluding that the Tail Suspension Test is sensitive only to type A MAOI but it can be noted that clorgyline, moclobemide and toloxatone are clinically active as antidepressants (Murphy et al 1979, Dencker and Nagy 1983, Casacchia et al 1984) whereas the antidepressant effects of pargyline are at best limited (Murphy et al 1979). In this respect, the results obtained with atypical antidepressants are pertinent because these compounds, in particular mianserin, while possessing demonstrated clinical efficacy, are not readily detected using classical pharmacological tests for antidepressant activity (Porsolt 1981).

Comparison with the behavioral despair test

The results obtained in the present experiments confirm those obtained in an earlier version of the Tail Suspension Test where individual results were analysed manually from smoked drum recordings of the animals' activity (Steru et al 1985a). Furthermore the findings with the Tail Suspension Test are largely consistent with those reported for the "behavioral despair" test where immobility is induced in rodents by forcing them to swim in water from which they cannot escape (Porsolt 1981).

In both tests immobility is decreased by antidepressants, psychostimulants and anticholinergics and increased by tranquilizing agents in particular neuroleptics. One important pharmacological difference is that antidepressants which selectively inhibit the uptake of serotonin (eg citalopram, indalpine) are clearly active in the Tail Suspension Test but not in the "behavioral despair" test. A further difference is that antidepressants are active in the Tail Suspension Test at doses considerably lower than those found active in the "behavioral despair" test. For example, imipramine significantly reduces suspension-induced immobility from 1 mg/kg i.p. (present experiments, Steru et al 1985a) whereas about 30 mg/kg is required to significantly reduce immobility induced by forced swimming (Porsolt et al 1977). The reasons for these differences are not clear but may be related to the marked hypothermia occurring during forced swimming (Porsolt et al 1979) or the presumably greater physiological stress induced in these conditions (Thierry et al 1986). Whatever the reasons, the fact that the Tail Suspension Test is sensitive to selective serotonin uptake blockers and in general to lower doses of test compounds represents an important advantage for this procedure as a primary behavioral screening test for antidepressants.

Utility of the power of movements parameter

A further factor of potential interest is the possibility, provided by the apparatus, of measuring the power of the movements emitted by the animal during the test. Although the available data are preliminary, there appear to be interesting differences between the compounds in their effects on this parameter. For example, while tricyclic antidepressants and most of the atypical agents tend to increase the power of the movements, the two serotonin uptake inhibitors had no effect, as was also the case with the MAOI. Although confirmatory data are required it is tempting to speculate that the absence of effect on this parameter might be due to the potent serotonergic stimulating properties or lack of noradrenergic stimulating properties which distinguish these compounds from the other antidepressants tested. Furthermore, while both minor and major tranquilizers increased the duration of immobility, they could be clearly distinguished in terms of their effects on the power of the movements. The decrease of power observed with diazepam and clobazam might well reflect the muscle relaxing activity of these benzodiazepines (Randall and Kappell 1973).

Ethical considerations

A final factor which cannot be ignored concerns the ethics of behavioral models, particularly those proposed for the testing of antidepressant drugs where by definition the animal is not rendered happy. The experimenter's aim in this kind of research should be to reduce the animal's discomfort to a minimum which is still compatible with the research goal. We have analyzed this aspect in detail elsewhere (Thierry et al 1986) and concluded that the Tail Suspension Test procedure appears to cause a minimum of suffering to the experimental animals and in any case considerably less than that induced in the traditional "behavioral despair" test (Porsolt 1981) or in "learned helplessness" paradigms (Sherman et al 1982) or even in the several variants of the reserpine test where the animals can remain under the influence of reserpine for several hours.

Conclusions

The automated version of the Tail Suspension Test described in the present paper appears to constitute a rapid, objective, sensitive and ethically acceptable screening procedure for detecting

antidepressant activity. There appear to be few false negatives and the availability of two behavioral measures, immobility and the power of the movements, suggest that this automated version of the test could also be useful for determining activity profiles for different kinds of psychotropic agents.

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Letter to the editor

The tail suspension test: Ethical considerations

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Is hanging a mouse or a rat by its tail for 6 min an act of cruelty? This is the subject of the following paper which analyzes certain physiological and behavioral aspects of the question. It seeks to justify the Tail Suspension Test (Stéru et al. 1985) as an improvement rather than a regression in the ethics of behavioral models of depression in rodents.

Haemodynamic imbalance

Everyone has experienced the discomfort of the head-down position but it is unlikely that rodents experience the same kind of discomfort. Basic knowledge of blood circulation has been established for a long time (Dow and Hamilton 1965). It is well known that human haemodynamics are different from those of other mammals. The upright position is the first difference: because of gravity, systolic blood pressure in humans varies from about 100 mm Hg in the aorta to about 50 mm Hg in the brain. Any lowering of the head demands a reequilibration of the blood pressure. Another factor is the large size of the human brain with its large diameter carotids. This allows a heavy blood flow which is balanced by the venous return. Any limit to this venous return unbalances the pressures, increasing the brain pressure and causing the discomfort known in the head-down position in man. Still another factor aggravating this discomfort in man is the large plasma volume which amplifies the consequences of any imbalance.

In contrast, the mouse lives in a horizontal rather than upright position. It is of small size and there would be little or no haemodynamic imbalance to cause stress when the mouse is placed in the head-down position. It seems unlikely, therefore, that the discomfort caused by tail suspension represents the stressing factor in this test.

Assessment of the stress factor

As the authors have previously reported (Thierry et al. 1984) the Tail Suspension Test can be considered as a derivative of the "behavioral despair" test (Porsolt 1981). In the latter test, the animal is placed in a cylinder containing tepid water (22–24 °C). For the animal, this presents a totally new and stressful situation because a laboratory animal has never known water other than in bottles. The animal first reacts by trying to escape. After a certain time the animal learns that no escape is possible and remains in a characteristic immobile posture just keeping its nose above the water.

The "behavioral despair" test has a side effect that is generally ignored, a severe hypothermia which can be measured at the end of the test (Porsolt et al. 1979). In general, the animal body's temperature tends to approach that of the water in which it is swimming. The most dramatic hypothermia occurs in mice, with rectal temperatures as low as 31 °C compared to a normal value of 37.5 °C after exposure to water at 23 °C for 6 min. Although the effect of this hypothermia is difficult to assess, it seems likely that the animal is submitted to a severe physiological stress; there is ample defecation, frequent squeaking and the slower motion observed at the end of the test is probably symptomatic of this discomfort. From a pharmacological point of view it is likely that the marked hypothermia could have an influence on drug activity, although the decrease of immobility by imipramine does not appear to be due to an antagonism of water-induced hypothermia (Porsolt et al. 1979).

In the Tail Suspension Test it seems reasonable to suppose the involvement of a stress factor considering the paradigm on which the test is based. We presume that the stress is caused by the restriction of motion; the animal is seen trying to escape but ceases after a few minutes. There is no hypothermia, however, and the animals show normal post-test behavior. The Tail Suspension Test itself does not appear to be painful; the animals are attached using an adhesive tape and squeaking is virtually never observed.

Another argument which indirectly supports the notion that the Tail Suspension Test is less stressful than the "behavioral despair" test is the fact that antidepressant drugs act at considerably lower doses in the former test. For example, imipramine reduces forced swimming induced immobility in mice at about 30 mg/kg IP (Porsolt et al. 1979) whereas a dose of 4 mg/kg IP is sufficient to induce a significant reduction of immobility in the Tail Suspension Test (Stéru et al. 1986). This greater pharmacological sensitivity could be taken to suggest that the physiological imbalance induced in the Tail Suspension Test is less severe than that occasioned by forced swimming.

Offprint requests to: R.D. Porsolt

Conclusion

The above analysis of haemodynamic, behavioral, physiological and pharmacological factors concord in suggesting that the Tail Suspension Test is considerably less stressful to experimental animals than the traditional "behavioral despair" test. It should always be borne in mind, however, that any attempt to model depression in animals by definition does not render them happy. The aim is therefore to reduce the animal's discomfort to a minimum which is still compatible with the research goal, the discovery of new antidepressant agents. This important ethical consideration, together with the greater pharmacological sensitivity of the procedure, suggests that the Tail Suspension Test is a useful addition to the battery of behavioral tests available for evaluating antidepressant activity in animals.

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What's Wrong With My Mouse?

Behavioral Phenotyping of Transgenic and Knockout Mice

Jacqueline N. Crawley, Ph.D.

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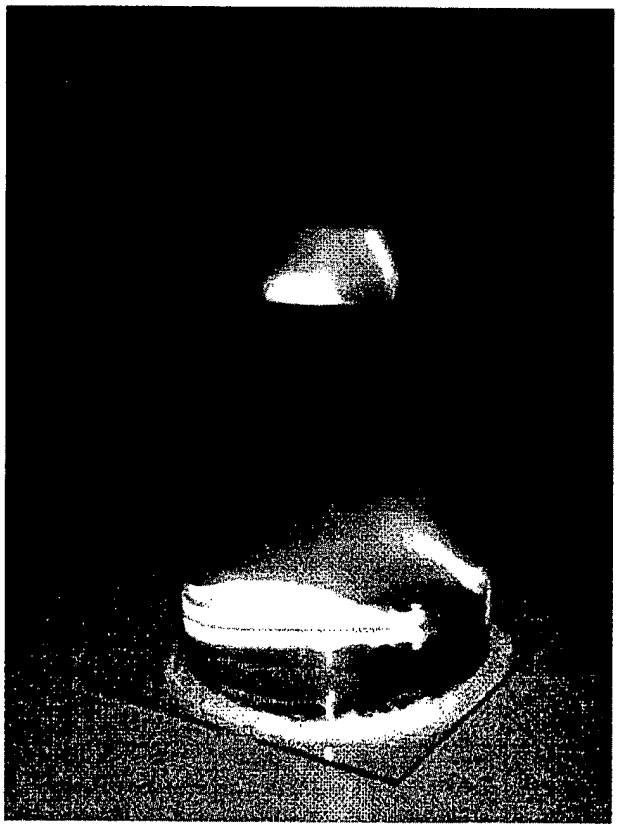
the elevated plus maze lies in the continuous nature of the runway. In the plus maze, mice will stay in the central start box and may repeatedly return to the start box rather than fully enter an arm. Start box behaviors introduce ambiguity in scoring an arm entry and increase the variability of the data. Handling of the mouse on repeated trials introduces an element of stress that may influence anxiety scores. The zero maze has no start box, and the session is continuous, obviating these problems.

Depression-Related Behaviors

The **Porsolt forced swim test** measures the time spent swimming versus the time spent floating in a tall cylinder filled with water (Porsolt et al., 1977, 1978; Drugan et al., 1989; Detke et al., 1995; Hansen et al., 1997; Redrobe and Bourin, 1998). For mice, the water is filled to a depth of at least 10 cm, which exeeds the distance to which the tail can extend, so the mouse cannot balance on its tail at the bottom of the cylinder. The top of the cylinder is at least 15 cm above the upper surface of the water, such that the mouse cannot climb out of the cylinder. Our laboratory uses a cylinder that is 20 cm in diameter and a waterline that is



(*a*)

FIGURE 10.8 *(Continued)*

Mean number beam interruptions

Percent time active

FIGURE 10.8 *(a) Porso*
(b) Stressors affect perfor
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test, as compared to mic
Hebert et al. (1998), p. 26

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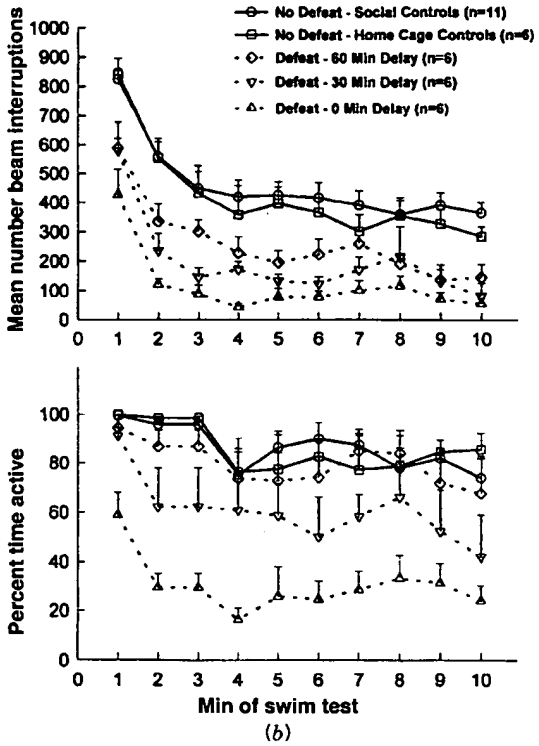


FIGURE 10.8 *(a) Porsolt swim task cylinder filled with water.* [Photograph contributed by the author.] *(b) Stressors affect performance on the Porsolt forced swim task. Mice previously defeated in a series of resident–intruder encounters showed higher levels of immobility, as measured in an automated swim test, as compared to mice that were undefeated or had no prior resident/intruder encounters.* [From Hebert et al. (1998), p. 262.]

15 cm above the bottom of the cylinder. The water is maintained at room temperature or warmer, up to 35°C. The mouse is placed in the water and undisturbed for the test session. Session durations between 4 and 20 minutes have been used for mice. A preexposure period of 2–5 minutes before a short test session has been used, with scoring beginning immediately thereafter. Mice will generally swim in the water, apparently seeking an escape route. After some time, the mouse may stop swimming and instead will float on the surface of the water, appearing to have "given up the search." Fatigue is a factor but does not appear to explain the cessation of swimming, since a minor disturbance will reactivate swimming. The investigator records the total number of seconds spent floating over the course of the test session. A water-filled cylinder used in the Porsolt swim test for mice, and representative data, are shown in Figure 10.8.

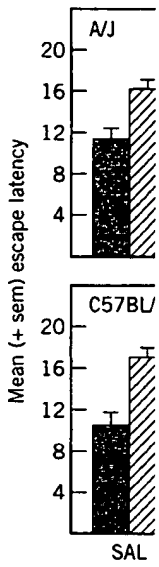
The Porsolt forced swim test is particularly sensitive to serotonergic antidepressant drugs (Borsini, 1995; Redrobe and Bourin, 1998). Treatment with antidepressants reduces the total time spent floating. However, other categories of psychoactive drugs have shown positive responses on the Porsolt forced swim test (Willner, 1984). In addition, the acute action of the antidepressant treatments in rodents on this test is inconsistent with the chronic

administration required for antidepressant drug action in treating human depression (Willner, 1984). A genetic component underlying the forced swim test in rats has been demonstrated by comparison of the Maudsley Reactive and Nonreactive strains (Overstreet et al., 1992).

Stressors such as immobilization and footshock enhance immobility in the Porsolt forced swim test (Weiss et al., 1981; Zebrowska-Lupina et al., 1990). In an interesting recent study, social defeat produced depression-like effects on the Porsolt swim test, as shown in Figure 10.9 (Hebert et al., 1998). DBA/2 male mice were paired with highly aggressive C57BL/6 male mice in a series of four encounters. Defeat induced reductions in swim time in the DBA/2 mice. Swim time versus immobility was quantitated in this study using a photocell array located under the transparent bottom of the cylinder, which detects beam breaks when the animal moved on the surface of the water (Hebert et al., 1998). This automated system appears to provide a useful technical advance for more accurate quantitation and higher throughput screening of drugs in the Porsolt swim test.

Learned helplessness is another test for failure to try to escape an aversive stimulus. Developed by Steve Maier at the University of Colorado, a standard method for rats employs three parallel wheel-turn boxes (Weiss, 1971; Maier, 1984; Drugan et al., 1984, 1985, 1989, 1997). A rat is placed in each of three identical small Plexiglas boxes, in which a grooved Plexiglas wheel is located in front of the rat. The apparatus is diagrammed in the cartoon at the beginning of this chapter. In the **escapable** shock condition, tailshocks are administered. The wheel is connected to the tailshock control mechanism. Turning the wheel switches off the shock generator. Rats quickly learn to turn the wheel to avoid the shock. In the **inescapable** shock condition, the first wheel is yoked to the second wheel, which is in front of the rat in the escapable shock condition. The rat in the inescapable condition receives the same number of shocks as the rat in the escapable condition but has no personal control over the delivery of the aversive stimulus. The rat in the **control** condition has the electrode connected to its tail, but the electrode is not connected to the shock generator. The control rat thus experiences no contingencies and receives no tailshock. At the end of a 1-hour session, the rats are returned to their home cages. Twenty four hours later, each animal is placed in a new apparatus, a shuttlebox. The task on the second day is the standard active avoidance. The animal moves from the left chamber to the right chamber of a two-chambered shuttle box, and back again, to escape or avoid footshock that is administered to the grid floor of one chamber at a time. Rats previously in the control and escapable condition perform well on the active avoidance task, receiving very few shocks. Rats previously in the inescapable condition perform poorly on the active avoidance task. Approximately half the rats trained in the inescapable condition never learn the active avoidance response. The failure to learn a simple escape task, 24 hours after exposure to an inescapable stressor, has been interpreted as behavioral despair. The rat appears to have learned that it is helpless to avoid stressors. Hymie Anisman and co-workers at Carleton University in Ottawa have adapted the inescapable shock paradigm for mice (Anisman et al., 1979; Shanks and Anisman 1989, 1993), as shown in Figure 10.9.

The **tail suspension** test has some of the characteristics of the Porsolt swim test and has been successfully used for mice in several laboratories (Stéru et al., 1985; Van der Heyden et al., 1987; Trullas et al., 1989; Vaugeois et al., 1996). The mouse is simply held by the tail, suspended dangling in the air. Some laboratories use adhesive tape to suspend the mouse for 6 minutes or more, after one or more training sessions, over one or more repeated tests



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Behaviors Related

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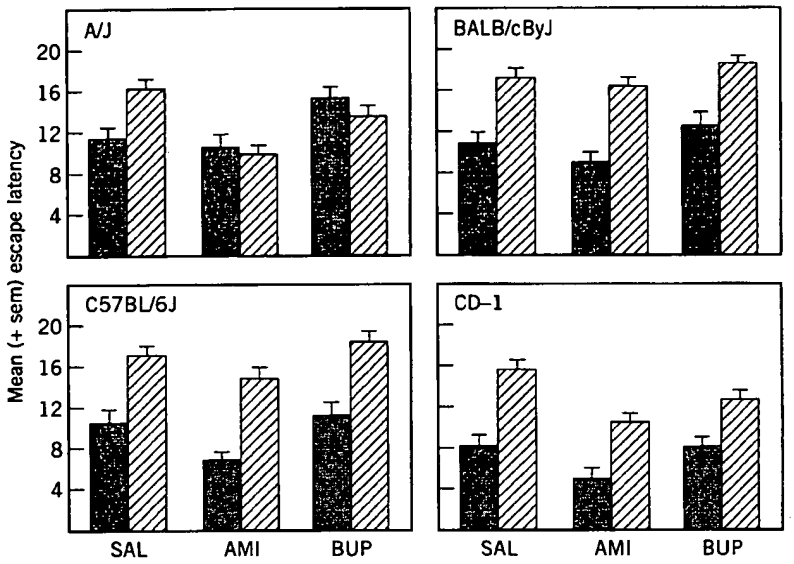

FIGURE 10.9 *Four strains of mice were tested after vehicle treatment (SAL) or 14 days of antidepressant drug treatments (amitriptyline, AMI, or bupropion, BUP), on escape deficits following an inescapable stressor. Mice receiving no footshock (black bars) showed faster escape in a shuttlebox escape task 15 days later, as compared to mice previously receiving inescapable footshock (striped bars). Antidepressant drug treatments significantly improved escape behavior in one strain (A/J) but not in the other strains (BALB/cByJ, C57BL/6J, CD-1).* [From Shanks and Anisman (1989), p. 124.]

(Vaugeois et al., 1996; Ukai et al., 1998). Cumulative immobility time is recorded by the observer or by measurement through a hook connected to a strain gauge (Vaugeois et al., 1996). The strain gauge transmits movements of the mouse to an automated unit that sums the total seconds spent immobile. Acute treatment with antidepressant drugs reduces immobility time (Stéru et al., 1985; Van der Heyden et al., 1987; Vaugeois et al., 1996). A genetic component underlying differences in immobility time was demonstrated in strain distribution studies of inbred strains of mice (Van der Heyden et al., 1987; Trullas et al., 1989). Selective breeding of high and low immobility scoring mice, shown in Figure 10.10, further indicates a genetic component to performance on the tail suspension task (Vaugeois et al., 1996).

Behaviors Related to Symptoms of Schizophrenia

Schizophrenia is the psychiatric disease that has been the hardest to model in animals. Possibly this is a uniquely human disorder. Guidelines for evaluating an animal model of schizophrenic-like symptoms for antipsychotic drug responsiveness are shown in Table 10.3. The majority of early tests for behaviors related to symptoms of schizophrenia were based on the antipsychotic activity of dopamine D-2 receptor antagonists (Ellenbroek and Cools, 1990; Higgins, 1998). Rodent behaviors induced by dopaminergic D-2 receptor agonists acting at the mesocorticolimbic dopamine pathway include **locomotor hyperactivity** and **stereotyped sniffing/grooming**. Dopamine, apomorphine, amphetamine, the D-2

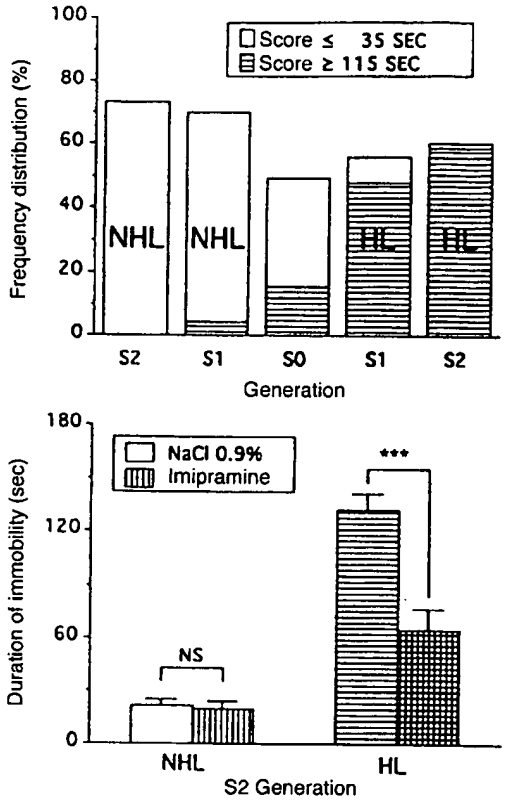

FIGURE 10.10 *Immobility in the tail suspension test was selectively bred from founder mice showing high versus low numbers of seconds of behavioral immobility. Treatment with an antidepressant drug, imipramine, reduced immobility time in the high scoring line of mice.* [From Vaugeois et al. (1996), p. R2.]

agonist quinpirole, and related drugs are administered systemically or through bilateral indwelling cannulae previously implanted into the nucleus accumbens. Locomotor activity in an automated open field is recorded over a 10- to 60-minute session. Higher doses of these drugs are administered in a separate experiment to quantitate stereotypy. Incidence of repetitive, stereotyped grooming, sniffing, and head movements are recorded every 15 seconds by an observer using a standardized stereotypy scoring system (Creese and Iversen, 1973). Drugs that block dopamine agonist-induced hyperactivity and stereotypy and are antagonists at the D-2 receptor represent good candidates for antipsychotics with a dopaminergic mechanism of action (reviewed in Ellenbroek and Cools, 1990; Higgins, 1998). This approach was useful in generating new D-2 antagonists that were biologically and behaviorally active. However, D-2 antagonists often exacerbated the negative symptoms of schizophrenia, and long-term treatment with D-2 antagonists sometimes resulted in the development of tardive dyskinesias (Breier, 1996). Current paradigms seek to expand beyond the dopamine hypothesis of schizophrenia by testing new classes of drugs on behaviors that are not directly activated by D-2 receptor ligands.

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Source: Ellenbrock and Cools